



PHILIP MORRIS

U.S.A.

RESEARCH CENTER

RICHMOND, VIRGINIA

2057725153

MONTHLY PROGRESS REPORT



0000008157

BAR CODE

92-001

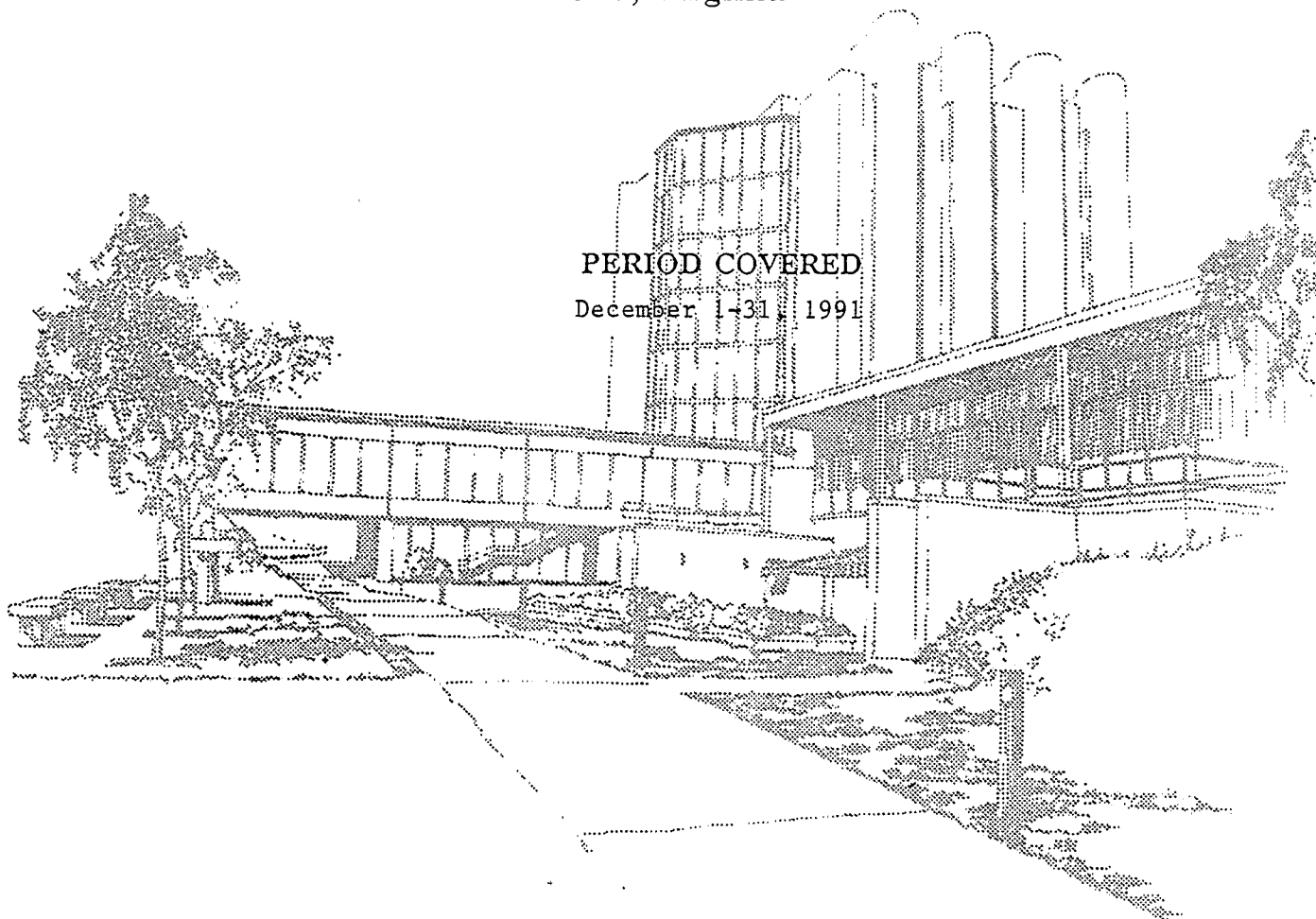
ACCESSION NUMBER

PHILIP MORRIS USA
Research Center
Richmond, Virginia

V. E. Willis
ISSUED TO

PERIOD COVERED

December 1-31, 1991



THIS REPORT IS CONFIDENTIAL TO THE BUSINESS OF THE COMPANY; IT HAS BEEN ASSIGNED TO YOU, IT IS NOT TRANSFERABLE AND MUST NOT BE PHOTOCOPIED.

IF THE REPORT HAS SERVED ITS PURPOSE AND IS NO LONGER NEEDED, PLEASE RETURN IT TO THE CENTRAL FILE AT THE RESEARCH CENTER FOR RECORD KEEPING AND DESTRUCTION.

DATE ISSUED: January 15, 1992

2057725164

DISTRIBUTION

Ms. D. J. Ayers
Dr. S. D. Baldwin
Mr. H. G. Burnley
Mr. W. T. Callahan
Dr. R. A. Carchman
Dr. J. L. Charles
Mr. R. A. Comes
Dr. K. A. Cox
Dr. R. H. Cox
Dr. R. W. Dwyer
Dr. C. L. Ellis
Dr. R. A. Fenner
Dr. R. N. Ferguson
Mr. E. B. Fischer
Mr. J. M. Garman
Ms. B. L. Goodman
Mr. H. J. Grubbs
Ms. B. M. Handy
Mr. C. R. Hayward
Mr. R. P. Heretick
Dr. K. S. Houghton
Dr. Y. Houminer
Mr. T. M. Howell
Ms. S. A. Hutcheson

Ms. R. W. Kanipe
Ms. R. D. Kinser
Dr. K. B. Koller
Mr. W. F. Kuhn
Dr. A. C. Lilly
Dr. D. B. Losee
Dr. R. W. McCuen
Mr. T. W. Murray
Mr. J. L. Myracle
Mr. S. B. Nelson
Dr. G. J. Patskan
Research Center Library
Dr. W. R. Raymond
Mr. C. H. Rowe
Mr. J. E. Schardt
Dr. K. H. Shafer
Mr. H. L. Spielberg
Mr. J. O. Stimler
Mr. G. Vilcins
Mr. D. C. Watson
Dr. J. F. Whidby
Ms. V. E. Willis
Mr. G. N. Yatrakis
The Central File

2057725165

Table of Contents

	<u>Page</u>
<u>PRODUCT DEVELOPMENT</u>	
2108 New Product and Filter Technology	1
2305 Applied Flavor Investigation	4
2306 Marlboro Standardization/International Support	6
2307 Basic Flavor Investigation	8
4016 Paper Technology	N/R *
4017 Paper Making Processes	10
4018 Paper Product Development	12
4022 Product Development, Affiliates and Licensees	N/R *
4030 Product Development, Export	N/R *
4031 Product Development, U.S.A.	14
5001 Packaging Studies	15
<u>PROCESS DEVELOPMENT</u>	
1309 Cast Leaf Development	19
1806 New Tobacco Processes	21
1810 ART Process Development	23
1811 Process Chemistry and Tobacco Fundamentals	N/R *
1812 New Expanded Tobacco	25
<u>RESEARCH</u>	
0008 Computer Applications Division	31
1101 Entomological Research	35
1620 Electrophysiological Studies	N/R *

2057725166

	Table of Contents (continued)	<u>Page</u>
1720	Analytical Microscopy	37
1757	Analytical Specifications	38
1761	Materials Evaluation and Infrared Investigations	39
1762	Molecular Structure Determination	41
1902	Tobacco Microbiology	42
1904	Tobacco Physiology and Biochemistry	45
2500	Inorganic Synthesis	47
2501	Smoke Chemistry	51
2520	Organic Synthesis	53
2526	Greenhouse Operations	55
2527	Radiochemical Services	58
2705	Tomorrow	59
2706	Tobacco Processing Principles	60
6502	Process Monitoring and Smoke Investigations	62
6503	Chemical Analysis	64
6505	Special Investigations	65
6902	Biochemical Special Investigations	66
6906	Biological Effects of Smoke	68
6908	Smoke Condensate Studies	69
6912	Tobacco/Smoke Relationships	71
8101	Cigarette Testing Services	72
	Program Index	75

* N/R = No Report

PRODUCT
DEVELOPMENT

2057725168

PROJECT NUMBER : 2108
PROJECT TITLE : New Product and Filter Technology
SECTION LEADER : W. T. Callaham
PERIOD COVERED : December, 1991

I. WEB FILTERS

- A. **Objective:** To characterize web-type filter materials and develop subjectively acceptable products with them.
- B. **Status:** Mono filters were produced with four webs made at James River - Neenah Technical Center which contain 100% softwood pulp. The four pulp types were subjectively screened in hand made cigarettes in preparation for a mill run in January, 1992.

Mono filters were produced with PM Web with/without Lecithin as a Yankee dryer release aid. The models were subjectively screened in hand made cigarettes in preparation for a mill run in January, 1992.

2mg models with CA tow, Tela/CA dual, PM Web/CA dual, PM Web + PZ/CA dual, CA Web/CA dual, CA Web + PZ/CA dual, and paper core concentric dual filters were produced in Semiworks. Analytical and subjective comparisons are currently in progress.

II. PRODUCT DEVELOPMENT SUPPORT

- A. **Objective:** To provide cigarette/filter design, modelling and testing support for major new product development programs.

Half Nic: Several hand made models have been given to Flavor Technology personnel for subjective evaluation. A modified PCC/CA filter was prepared by removing the middle section of the paper core and filling it with SCCW carbon.

Project 41: Cigarettes were made from PCC filters with wood/tobacco carbon cores received from American Filtrona Company for testing. Initial tar deliveries were high and cigarettes will be remade for Danchi panel testing.

Carbon Recycled from MULFI Combiners: Carbon-on-tow inner filter components using recycled MF-2C carbon were made at Semiworks. A request for factory made Parliament 100 cigarettes using the above inner filter components was submitted to Stockton Street.

2057725169

III. ALTERNATIVE PLASTICIZERS

- A. **Objective:** Qualify alternative plasticizers which offer a product advantage.
- B. **Status:** Two test models (TEC w/22% menthol at 8% application rate + 25mg/pack MOF, and TEC w/ 22% menthol at 11% application rate only) and a control Marlboro Menthol KS were made in Semiworks. After two weeks aging in laboratory conditions, these models were submitted to CTSD for menthol in smoke analysis. Further laboratory and accelerated aging studies are ongoing to assess the effects on menthol delivery and subjective response.

Marlboro KS cigarette models using 50/50 mixtures of triacetin and triethyl citrate were submitted to the Richmond Panel for subjective analyses.

IV. CARBON TECHNOLOGY

- A. **Objective:** Conduct fundamental studies of carbons and carbon filtered cigarettes to better understand their performance.
- B. **Status:** Factory trial testing of non-impregnated SCCW was completed this month and the data is being analyzed. Carbon dust was collected daily from the plug-space-plug combiners. Dust will be collected from the control SCCW run on the same two combiners. The total dust for 60 drums of test carbon will be compared to that for 60 drums of control carbon.

V. MENTHOL TECHNOLOGY

- A. **Objective:** To develop alternative mentholation techniques which offer a product advantage.
- B. **Status:** Menthol-in-Dope (MID) rods were received from Eastman and submitted to CTSD for menthol analysis. The menthol content of 7.55 to 8.83mg/plug was within the target ranges. Cigarette making and packing have been requested for Marlboro Menthol Lights and Full Flavor test models with the MID filters. Control models will use standard FT-107 tow and mentholated foil packing. Making and packing of all of these models is scheduled for the week of January 6.

VI. PRECOATED POROUS COMBINING WRAP

- A. **Objective:** To develop a combining wrap for cavity filters which reduces the ventilation variability and increases the ventilation capability.
- B. **Status:** A meeting was held to plan a January evaluation run of Lark Super Lights KS at 6mg tar delivery using three porous combining wraps.

VII. MACHINERY DEVELOPMENT

- A. Objective: Develop or modify manufacturing equipment to support Product Development efforts.
- B. Status: An inspection and demonstration of the moving orifice coater and conveyor was held at Jewett's Automation. Additional guarding for nip points is indicated and additional work is required on the electrical controls to make them fully operational. Installation of this equipment in the Filter Development Lab is scheduled for the week of January 6.

Work is proceeding at Fox Valley Ltd. on the 12" laboratory coater. Fabrication of the drying drums is almost complete and the bobbin unwind system is finished. Work requests have been initiated for installation of an exhaust system for the coater.

PROJECT NUMBER : 2305
PROJECT TITLE : Applied Flavor Investigation
PROJECT LEADER : J. Swain
PERIOD COVERED : December, 1991

I. PROCESS SUPPORT

- A. **Objective:** To develop and evaluate process modifications to maintain subjective and physical quality.
- B. **Status:** RCB Test 6 from 350 fpm linespeed trials was selected for POL 03012 evaluations in Marlboro. Dryer temperature profiles on Line 1 were increased mainly in zones 4-9 to achieve drying and subjectives similar to the RCB control (330 fpm). POL 03014 of Marlboro cigarettes with unwashed replacing washed Burley stems in RCB will be remade due to low tar delivery. LTRL trials with European feedstocks and the TC flavor system were completed. Park 500 analyses of the six test LTRL's have been completed prior to initial subjective evaluations to be completed in January.

II. CAST LEAF PROGRAM

- A. **Objective:** To develop and evaluate flavor systems for cast sheet utilization.
- B. **Status:** Cast Leaf Panel evaluations have been initiated for Marlboro cigarettes with 24% BL levels from pilot trials of slurry aging times and dryer profiles. Comparisons are being made to production RCB to qualify the Cast Leaf Pilot conditions.

Cigarette analyses have been completed and subjective evaluations initiated for Spanish Marlboro with ASTA replacing RCB and SIVA replacing RLB in Spanish Marlboro and Chesterfield cigarettes.

III. NET PROGRAM

- A. **Objective:** Provide Flavor Technology Support for the New Expanded Tobacco Program.
- B. **Status:** A series of test BLDET individual and blended C34 Burley and #10 Bright trials were made for subjective evaluations of NET versus DIET processing. Uncased and only C34 Burley cased BLDET trials of NET were made to different exit tower OV's for comparisons.

IV. DRY FLAVOR REPLACEMENT

- A. **Objective:** To develop, evaluate and establish specifications for dry flavor replacements.

- B. Status: Flavor formulation and specifications have been transmitted for implementation of the dry flavor replacement at the BL Plant. The Flavor Center produced flavor 75-700 for the initial two months of production in preparation for implementation.

V. PROJECT GRAIN

- A. Objective: To significantly reduce alcohol levels in PM flavor systems, while maintaining product subjective integrity.
- B. Status: Internal panel evaluations of B&H menthol full-flavor cigarettes with 100% reduction of alcohol in the BTC showed no obvious differences. Evaluations of similar changes in B&H Lights menthol cigarettes are in progress and tests of alcohol removal from the aftercut have been requested.

A factory trial of the modified BTC without alcohol addition has been completed at 20th Street on export strip production. On-site monitoring showed no obvious problems and preparations have been made to evaluate the use of a BTC concentrate at the McKinney facility in January.

POL 03005 of Marlboro cigarettes with a 52% total alcohol reduction has been shipped to panelists. The test of Marlboro cigarettes with rearranged humectants (POL 03015) to achieve similar alcohol reductions will be remade in the Semi-Works.

PROJECT NUMBER : 2306
PROJECT TITLE : Marlboro Standardization/International Support
SECTION LEADER : V. E. Willis
PERIOD COVERED : December, 1991

I. MARLBORO STANDARDIZATION

- A. **Objective:** Analytical and subjective evaluations of Marlboro KS/LS factory production.
- B. **Status:** The October 9, 1991 factory pick-up was completed. Marlboro (KS and FTB) and Marlboro Medium (KS) were picked-up on November 26, 1991. Park Manufacturing Facility was also included in this pick-up (MF KS). Analytical and subjective evaluations are in progress.

II. DOMESTIC SUPPORT

- A. **Objective:** Provide subjective evaluations (rod aroma and smoking characteristics) for domestic markets.
- B. **Status:** Twenty-five (25) panels have been completed during this reporting period. The Cast Leaf Panel training continues.

III. INTERNATIONAL SUPPORT

- A. **Objective:** Provide subjective evaluations (rod aroma and smoking characteristics) for the international markets.
- B. **Status:** Six (6) panels were completed during this reporting period.

Subjective evaluation of Phase Two of the Panama Food/Tobacco Distribution study continues.

IV. INTERNATIONAL

- A. **Objective:** To provide flavor and subjective support to the International development groups as needed.
- B. **Status:**

Project 41 (Japan): Flavor Technology subjectively evaluated Danchi Test E-V941. The cigarettes evaluated were from the market place and included Merit Lights, Mild Seven Super Lights, Frontier Lights, and Caster Mild. The Richmond Panel subjectively approved the test and it was released for shipment.

Flavor Technology and the International Panel subjectively evaluated a series of nine prototypes for the selection of 4 mg models for a future Danchi test. Three fillers and three filters were evaluated. A recommendation was made to test Blend 244 with each of the three filters and Blend 298 with filter 1 (dual carbon on paper).

Lark/Lark Milds Diagnostic Series (Japan): Flavor Technology subjectively evaluated the remake of PMI (monadic) Test E-V312. The cigarettes included Lark Milds at 10 mg TIOJ tar with Starship blend and flavors, Lark Milds (10mg TIOJ tar) with Starship cased blend and Lark aftercut, Lark Milds SP (factory), and L&M Milds (cork-tipped). The testing format for this PMI was redesigned. Richmond Panel subjectively approved the test and it was shipped.

Export Product Development (Taiwan): Flavor Technology subjectively evaluated PMI Test E-V780. The cigarettes evaluated were B&H Lights 100 FTB (M/C production) and YSL 100 FTB (market place). The Richmond Panel approved the test and it was released for shipment.

Export Product Development (GCC/Iran): Flavor Technology subjectively monitored the Stockton Street start-up of Philip Morris 100 SP (for GCC). All product evaluated from December 17, 1991 to December 20, 1991 (C shift) was subjectively acceptable. Two shifts of Merit 100 SP (for Iran) (December 16, 1991-B & December 17, 1991-C) were also subjectively acceptable.

Merit Lights Program (Hong Kong): Flavor Technology subjectively evaluated PMI Test E-T714. The cigarettes included Kent KS SP from the Hong Kong market and a Merit prototype at 8 mg UK tar (U.S. Merit filler, Merit Lights Japan charcoal plug with white tipping). The test was approved by the Richmond Panel and released for shipment.

PMI Testing (Hong Kong): Flavor Technology subjectively evaluated PMI Test E-T712. The cigarettes evaluated were Marlboro Lights at 9 mg UK tar with cork-on-white tipping and Marlboro Lights at 9 mg UK tar with white tipping. The test was evaluated by the Richmond Panel and released for shipment.

Pan Asian Menthol (Hong Kong): Flavor Technology subjectively evaluated HKCP Test E-V814. The cigarettes evaluated included Salem KS and Salem Lights KS from the Hong Kong market and two prototypes made with PMSL filler at 8 mg UK tar, 0.60 mg menthol in smoke (target) and 14 mg UK tar, 0.45 mg menthol in smoke (target). The cigarettes were subjectively approved by the Richmond Panel and shipped.

PROJECT NUMBER : 2307
PROJECT TITLE : Basic Flavor Investigation
PROJECT LEADER : R. W. Hale
PERIOD COVERED : December, 1991

I. ANALYTICAL SUPPORT

A. Objective: To provide analytical support for activities related to development and application of flavoring materials.

B. Results:

Two hundred sixty-one (261) new samples were submitted this month, and three hundred ten (310) samples were completed. A backlog of sixty-one (61) samples was recorded at the end of the month.

1. Project 2304:

Twenty-six (26) samples, including flavor revisions, vendors samples and flavors for purity, were analyzed.

2. Project 2305:

Seventy (70) samples from Project Grain, ASTA, RCB, Burley Stems and flavors were completed.

3. Project 2306:

Eighty (80) samples, including filler, cigarettes and flavors, were analyzed.

4. Project 5001:

Seventy-five (75) samples of packaging materials were analyzed for residual solvents and GC/MS for unknown components.

5. Sixty-one (61) samples for projects outside of the Flavor Technology Division were analyzed.

II. FLAVOR INVESTIGATION

A. Objective: To develop new basic and applied flavor technology in support of new product development objectives.

B. Results:

1. Identification by GC/MS of the impurities isolated from glycerin/triacetin is ongoing.

2. Work has started on procedures to determine analytically and subjectively the components in the waterborne ink systems that are detrimental to our products.

PROJECT NUMBER : 4017
PROJECT TITLE : Paper Making Processes
PROJECT LEADER : R. M. Rogers
PERIOD COVERED : December, 1991

I. LOW SIDESTREAM SMOKE PROJECT

- A. **Objective:** Develop proprietary cigarette papers for low sidestream smoke.
- B. **Results:** Three Superslims paper trials were conducted at KC's Spotswood mill this month; 8.5, 10.5 and 12.5 Coresta with 10.5% mono potassium phosphate (MKP). A fourth sheet at 10.5 Coresta and 10.5% MKP but higher water extractable MKP was also attempted. KC has determined that sidestream smoke reduction is a function of water extractable MKP, the lower the extractable content, the higher the sidestream smoke reduction. The concentration of extractable MKP is determined by the reaction of MKP with CaCO_3 which occurs during the papermaking sizing process. Size solution temperature and the post size-press dryer temperature were identified as the two main process variables. Due to the problems in maintaining the MKP solution's solids content, only post size-press dryer temperature was used to manipulate water extractable MKP. KC indicated that the difference in the level of water extractable MKP in the two sheets at 10.5 Coresta may not be as large as desired.

Five Superslims papers (47.5 g/m^2 , 10 Coresta, 33% CaCO_3) utilizing five different types of calcium carbonate (Albacar, Microna-3, Microwhite-25, Multifex, and a combination of Microna-10 and Multifex) were produced at the University of Maine. Sheet properties (formation and general appearance) of all five papers are acceptable. The papers were produced to study the effect of ground versus precipitated calcium carbonate on cigarette performance properties such as sidestream reduction and puff count.

- C. **Plans:** Support production of pilot cigarette paper at Maine.

II. BANDED PAPERS (TOMORROW)

- A. **Objective:** Incorporate cross directional bands of fiber and/or filler in cigarette papers in order to vary cigarette burn rate.
- B. **Results:** Cellulon slurry (1% solids with 10% CMC) was printed on conventional cigarette paper using a modified gravure cylinder. Reasonably good band definition was achieved but wrinkles and creases developed during drying, apparently caused by the high amount of water used. Additional trials will be conducted to produce a sufficient quantity of paper for machine made cigarettes.

Bands of Konjac flour were printed on pre-sized commercial cigarette paper (137-1). Performance appears to be effective at a relatively low level of application (1.5 g/m^2) but the commercial viability of this material is questionable due to a high degree of tack and difficulty in printing.

The moving orifice and conveyor assembly was demonstrated at Jewett's machine shop. The assembly should be operational by the first week of January after installation of safety guards and modification of the control panel.

- C. Plans: Initiate evaluations of cellulosic materials for band application.

III. FILTER WEB DEVELOPMENT

- A. Objective: Develop a proprietary filter material in web form.
- B. Results: A number of pilot filter web runs were completed at James River's Neenah Technical Center. Tela web replacements were produced using three northern bleached softwood pulps (Terrace Bay, Thunder Bay, and Prince George) versus a control of Marathon pulp. All three pulp sources are talc free and give a subjective response similar to Marathon, which is equivalent to Tela. Terrace Bay (produced by Kimberly-Clark) had been chosen for use in both James River's Tela replacement and PM web.

A second set of runs was conducted to evaluate methods that would reduce Yankee adhesion as an approach to decrease PM web's propensity to lint. Use of a release agent applied directly to the Yankee (lecithin, a soy bean extract) reduced adhesion and appeared to reduce lint (at the doctor blade and on the surface of the sheet). The reduction in adhesion was proportional to the amount of lecithin applied. Reducing CB-10 dispersant from 2% to 1.5% also reduced adhesion on the Yankee without any observable decrease in formation. Reducing headbox pH (as low as 4.9) has no effect on Yankee release. Based on positive subjective evaluations, the use of lecithin as a release agent appears to be the best choice. Web trials (PM web and Tela replacement) are scheduled for the early January.

Two runs utilizing Fibrids at 15% and 20% were also completed. Dispersion was the major equipment limitation during these trials.

- C. Plans: Complete web trials at James River's Gouverneur tissue mill.

PROJECT NUMBER : 4018
PROJECT TITLE : Paper Product Development
PROJECT LEADER : B. L. Goodman
PERIOD COVERED : December, 1991

I. REDUCED SIDESTREAM CIGARETTES

A. Objective: Develop subjectively acceptable cigarettes with reduced sidestream smoke.

B. Results:

Singlewrap Superslims: Cigarettes were made in Louisville with papers containing two different levels of water-extractable potassium phosphate. The papers were coated in Kimberly-Clark's laboratories. This arose from work done by Kimberly-Clark in an attempt to explain why some previous Superslims papers have given higher sidestream reductions. Analysis of cigarettes and paper samples is under way; preliminary testing on the 8-port instrument indicates that the sidestream reduction for the two papers is different.

A millrun quantity of paper containing the PM-approved wet strength aid has been completed by Kimberly-Clark. The paper was run on their #14 machine in a one step operation at three different Coresta levels. Sample bobbins have been shipped for making test cigarettes in Louisville. Production paper is still made in the two step operation, and analysis is being done by QA and R&D.

Magic: Cigarettes were made in Semiworks with the new set of Ecusta magnesite papers sized with a 10% solution of K_2 succinate. The binary papers were analyzed as having 4.5% K_2 succinate; the ternary paper was analyzed as having 5.5% K_2 succinate. Models were made with the higher delivery filter construction using all six papers; additional models were made with the current filter system and with charcoal filters using three of the papers (55 g/m² with 20% $MgCO_3$ /20% $CaCO_3$, 45 g/m² with 25% $MgCO_3$ /15% $CaCO_3$, 45 g/m² with 18% $MgCO_3$ /4% $Mg(OH)_2$ /14% $CaCO_3$). Visibility reductions averaged 58% for the binary 55 g/m² models, 53% for the binary 45 g/m² models, and 58% for the 45 g/m² models. One model with the binary 45 g/m² paper and Merit Ultima filler was screened subjectively and found to be worthy of further work. This model burned very fast and consequently gave lower visibility reduction (42%).

An adhesive study was conducted in Semiworks using the 55 g/m² binary magnesite paper sized with 10.7% K_2 succinate solution. Cigarettes made with the test paper and a production control, each at two rates of sideseam adhesive application, were compared subjectively. There was no significant difference in tar delivery and puff count between the cigarettes at the adhesive extremes for either the test or the control papers. Subjective screening did not indicate any difference in taste between cigarettes at the two adhesive levels on the magnesite paper models.

Handmade models with paper made from non-solgel magnesium carbonate, an Ecusta magnesite paper, and a handmade Marlboro Lights were subjectively screened. The results indicate that further work is justified with the magnesium carbonate paper.

- C. **Plans:** Continue monitoring Superslims papers for Coresta values and additive levels, and monitor sidestream visibilities.

Analyze and evaluate cigarettes made with millrun paper from Kimberly-Clark's #14 machine.

Evaluate the most recent cigarettes made with magnesite papers, and continue development of an improved product.

II. PAPER SPECIFICATIONS

- A. **Objective:** To develop cigarette wrapper specifications for new product development and support of current products.

- B. **Results:** FTC smoking was completed on cigarettes made to compare actual tar deliveries with predicted deliveries from the Response Surface Study model. The actual tar deliveries agreed closely with predicted deliveries for most of the cigarettes produced. The tar deliveries of several cigarettes were different from predicted tar deliveries; all of these had paper at the higher levels of porosity in the set of models. Additional work will be done with the modeling statistics to try to determine if the model is accurately accounting for the effect of paper porosity.

- C. **Plans:** Perform additional regression correlations on the Response Surface Study delivery data.

Resubmit additional cigarette samples for FTC smoking to replicate the data for further testing of the Response Surface model.

PROJECT NUMBER : 2304/4031
PROJECT TITLE : Product Development, U.S.A.
SECTION LEADER : C. B. Altizer
WRITTEN BY : G. N. Yatrakis and J. L. Spruill
PERIOD COVERED : December, 1991

I. LOW TAR/HIGH FLAVOR

PROJECT BOLD

- A. **Objective:** Develop 1mg 85mm and 2mg 100mm regular and menthol products competitive with Now and Carlton.
- B. **Status:** Sell-in samples for Merit Ultima (four packings) were completed in Cabarrus. National production start-up began January 6 for soft pack versions with box versions beginning January 13.
- C. **Plans:** Monitor National start-up analytically and subjectively.

II. PROJECT MARLBORO

- A. **Objective:** Design and develop Marlboro products as possible line extensions.
- B. **Status:** 100mm models are being requested at 12mg and 13mg tar for analytical and subjective evaluations.
- C. **Plans:** Complete prototype samples for evaluations and address factory trial status for the line extension.

III. BRAND EXTENSIONS

- A. **Objective:** Design and develop extensions and modifications to existing brand families.
- B. **Status:**
B&H KS: POL 4003 (FF, Menthol) was released. Replicate tests 0497 and 4002 are scheduled for first quarter testing.
- C. **Plans:** Prepare preliminary specification for the line extension.

PROJECT NUMBER : 5001
PROJECT TITLE : Packaging Studies
PROJECT LEADER : B. Mait
PERIOD COVERED : December, 1991

PACKAGING STUDIES

A. **Objective:** Provide technical packaging support to Manufacturing, Manufacturing Services, Engineering, Purchasing and Quality Assurance. In addition, assist New Products Directorate in evaluating new packaging concepts and products.

B. **Status:** Residual solvent levels were determined on twenty-five packagings.

Nineteen samples were subjectively evaluated for Purchasing Technical Services (PTS).

The packaging material used on Merit Ultima was analytically and subjectively approved.

Plant tours of two suppliers, Sun Chemical and Thiele-Engdahl, were made in December to investigate the possibility of supplier partnering.

PROCESS
DEVELOPMENT

2057725184

PROJECT NUMBER : 1309
PROJECT TITLE : Cast Leaf Development
PROJECT LEADER : G. Gellatly
PERIOD COVERED : December, 1991

CAST LEAF DEVELOPMENT

- A. **Objective:** Develop subjectively and physically acceptable reconstituted tobacco sheets for domestic and international application using Cast Leaf technology and proprietary binder systems.
- B. **Results:** **Cast Leaf Pilot Plant** - Some modifications remain to be made to improve operational stability. However, none of these are interfering with the trial program. Forty-eight trials have been run since plant startup to qualify pilot BL and NBL subjectively with production RCB, to reduce tar delivery by DAP reduction and to reduce plant emissions by ammonia reduction. Trials were also begun to identify processing conditions using guar gum as a binder.

Pilot plant BL product using the same formulation and the same feedstock as the BL plant was shown to have similar physical properties to production RCB. The physical properties of NBL was shown to have superior physical properties to production RCB and have equal or greater strength than RL. NBL sheet with reduced DAP (4.0% vs. 7.5%) content, to reduce the tar delivery of NBL, had similar physical properties to production RCB.

The Williams grinder installation was completed and will be started up in January.

BL Plant Capacity Increase - RCB test sheets from the November line speed increase trials (at 350 fpm versus 330 fpm standard) were subjectively screened to determine the best dryer temperature profile. The selected test RCB showed no subjective difference on Flavor Development panels and is scheduled for POL evaluation.

Alternate Sheet Suppliers - RLTC type test sheets were produced at LTR using PME feedstocks. Sheets were made using combinations of two different burley stem washing techniques and two evaporator types in order to isolate and eliminate the cause of hot peppery subjective notes.

Test materials were forwarded to PME for TLA analysis and subjective screening.

C. **Plans:**

1. Complete the Cast Leaf pilot plant punch list.
2. Evaluate the Cast Leaf pilot plant dryer model.
3. Determine the LTR process mode producing subjectively acceptable RLTC type sheet.

4. Complete the subjective and survivability characteristics of SIVA and ASTA product from August Cadiz trials.
5. Determine the conditions of OV, packout temperature, and packing density necessary to induce ASTA product darkening in the laboratory.
6. Begin analytical work on European feedstock.

PROJECT NUMBER : 1806
PROJECT TITLE : New Tobacco Processes
PROJECT LEADER : C. A. Wood
PERIOD COVERED : December, 1991

PRIMARY IMPROVEMENT PROGRAM

- A. **Objective:** Identify process and equipment modifications to provide a highly flexible primary operation capable of producing filler with improved quality and filling power at a lower operating cost.
- B. **Status: Process Development** - An accelerated schedule for the New Primary Process has been developed and a task force has been selected to address major areas of responsibilities. A one year delay in the Cabarrus expansion project will provide an opportunity to impact the plant performance with the NPP design. A prefunding request has been approved and Semiworks reconfiguration to the NPP process was initiated over the holiday shutdown. A commitment to Hauni for delivery of the Direct Cylinder Conditioner for Semiworks was made the week of December 16.

Burley Drying Studies - Burley tobacco drying studies are ongoing to relate subjective and analytical characteristics to drying methodology. Blended burley with burley spray applied is being evaluated in a tray dryer over a range of drying condition temperatures, moistures, and time. Additional testing is being conducted on the Semiworks P&S apron dryer.

Cut Filler Delivery - The Flow Thru Hopper is currently being evaluated in baseline studies on a Mark 9 machine in Semiworks versus a standard machine. Filler quality and cigarette performance information is expected by the end of January.

Cigarette Weight Reduction - Data has been collected on the first series of tests for cigarette physicals and cut filler quality. Testing is underway to determine rod to rod chemical variations when blending cut components versus standard total blend processing.

New Primary filler will also be evaluated on the Flow Thru Hopper to determine the impact of improved filler and maker hopper combination.

DIET Temperature - Preliminary testing to determine if expanded tobacco suffers filling power collapse when added to warm, moist filler indicated improved cylinder volume of the blended filler when expanded tobacco was added to cool filler. Cigarette firmness data was inconclusive due to excessively noisy data and produced no significant trend. Current data is being used to establish a statistical design for further testing to quantitate filling power benefits.

- C. **Plans:** Accelerate the Semiworks NPP equipment installation to be completed by September 1992 with interim studies being conducted as the process is phased into production according to the task force program planning.

Continue burley drying studies in Semiworks and evaluate the tray drying results for definition of operating windows of temperature, moisture and time versus subjectives.

Cut filler delivery results will determine whether a unit will be recommended for factory trials to improve cigarette weight and firmness relationships and evaluate machine durability for an extended time.

Cigarette weight reduction data collection and analysis will provide further direction in process definition and priorities for the NPP program.

DIET temperature testing will continue based on the statistical test plan.

PROJECT NUMBER : 1810
PROJECT TITLE : ART Process Development
PROJECT LEADER : D. R. Fox
PERIOD COVERED : December, 1991

I. LIQUID ABSORBENT TREATMENT PROCESS DEVELOPMENT

- A. **Objective:** To develop separation, utilization, and disposal processes for the liquid absorbent effluent from the second-generation ART extraction process.
- B. **Results:** A bench-scale system run was completed to determine the maximum possible feed strength for the activated sludge process using primary effluent from Park 500 to dilute the spent absorbent. As previously noted, the microbial population appears to be healthier with the primary effluent in the feed compared to earlier runs in which deionized water was used to dilute the absorbent. This is presumably due to the more varied food materials in the primary effluent.
- C. **Plans:** The bench-scale system will be shut down over the holidays. It will be restarted in a cold room to simulate low-temperature conditions in January.

II. BL PLANT WATER TREATMENT

- A. **Objective:** Identify and develop techniques for reducing discharge levels of various constituents from exhaust air streams at the BL Plant and Richmond Stemmyery.
- B. **Results:** Additional checkout runs with the pilot Sequential Batch Reactor were completed in preparation for the startup of the unit in January. A test plant was developed with the assistance of McNamee Advanced Technology, our consultant for this project.
- C. **Plans:** Startup of this system is expected in mid-January.

III. BL PLANT AIR TREATMENT

- A. **Objective:** Identify and develop techniques for reducing discharge levels of various constituents from exhaust air streams at the BL Plant and Richmond Stemmyery.
- B. **Results:** The pilot adsorption system was installed on the roof of the BL Plant for treating a sidestream from the main dryer exhaust. Preliminary checkout runs were made to identify operational problems. The tubing leading to the adsorber is being traced and insulated over the holiday shutdown to eliminate condensation in the line from the cold outdoor temperatures.

Development Engineering is working on the design for a pilot water scrubber for the same air stream. This unit will be a packed scrubber of 1 foot diameter and 8 foot height with associated instrumentation.

- C. **Plans:** Testing of the adsorption system with XAD-4 for nicotine removal will begin at the first of the year. Evaluation of other adsorbents will be conducted later in the first quarter. The design for the pilot scrubber will be completed in January, with the system installed and ready to operate by the end of February.

PROJECT NUMBER : 1812
PROJECT TITLE : New Expanded Tobacco
PROJECT LEADER : E. B. Fischer
PERIOD COVERED : December, 1991

I. BATCH GASEOUS CO₂ IMPREGNATION

- A. **Objective:** Define process parameters for a batch gaseous CO₂ impregnation process.
- B. **Results:** Tests evaluating a small scale Witte separator to cool the expanded NET product prior to the humid-air reordering step were run this month. The test objective for this phase of the evaluation was to quantify size degradation across the unit at air velocities sufficient to separate stems from the expanded filler at low moisture content exiting the tower. Initial sieve results taken before and after the Witte showed no significant loss of size across the unit. Additional tests are planned to confirm these results.

Further testing of the Bermuda Hundred impregnator bulk feeder were run this month following modifications to the infeed of the bulker. Temperature profiles through the impregnated bed of tobacco continue to show no indications that this manner of loading the tobacco adversely affects the uniformity of CO₂ flow through the bed. Additional tests on this unit are planned at BHPF during the week of January 13.

Results from a second cigarette making study designed to quantify tobacco weight savings resulting from substituting NET product for DIET in a Marlboro cigarette at target firmness were completed. CV and sieve results consistently showed the NET product to have higher CV's and significantly more longs than the MC DIET counterpart. Based on the filling power increase of the NET product, previously established modelling techniques project a weight reduction of approximately 9 mg in a Marlboro type cigarette with 12% NET in the blend. However, efforts to experimentally demonstrate this weight savings have not been successful due to the high variability in firmness vs weight results relative to the expected 9 mg weight reduction. Experimental procedures are being reviewed to identify better techniques to establish this weight reduction potential experimentally in lieu of the calculated 42 comparison runs required for statistically significant data at the current variability level.

- C. **Plans:** Implement and complete the tests described above.

II. CONTINUOUS IMPREGNATION PROCESSES

- A. **Objective:** Develop a continuous impregnation process to improve the subjectives of expanded tobacco while maintaining equivalent cigarette filling power to the existing process.

- B. **Results:** All four ball valves were disassembled and inspected. Three were found to be in good condition. The seats and ball surfaces were relapped and the valves returned to service. The ball surfaces and the seats of the fourth valve were significantly scored. A manufacturing defect appears responsible for this failure. This valve was returned to Mogas for repair under warranty.

A test program, designed to study the impregnation process, has been completed.

- C. **Plans:** Begin the test program and optimize the basket design to obtain uniform impregnation of tobacco.

III. EXPANSION AND REORDERING PROCESSES

- A. **Objective:** Define alternate means of puffing, drying, setting, and reordering impregnated tobacco to improve product subjectives and physical characteristics relative to the present DIET process.

- B. **Results:** Moisture uniformity has been improved by introducing conditioned air into the zones exterior to the stack in the spiral reordering machine. The rotary valve installed at the spiral exit has successfully reduced air turbulence at that point without causing measurable product degradation.

The vibrating cooler at the tower exit was replaced with a perforated tray cooler. This unit successfully cooled the tobacco from about 210°F to 90°F in 50 seconds without the breakage observed in the vibrating cooler. No CV loss occurred in this initial test.

- C. **Plans:** Continue testing sealing arrangements of the spiral reordering unit to resolve the necessity of adding extra air to the zones. Continue evaluation of cooling conveyor to minimize residence time and determine product quality impact, if any.

IV. CHEMICAL STIFFENING

- A. **Objective:** Define a process to chemically stiffen expanded tobacco which will reduce thermal treatment and the associated subjective degradation while maintaining cigarette filling power equivalent to the current process.

- B. **Results:** Project on hold pending manpower availability.

- C. **Plans:** Complete patent application and file. Postpone further development work until manpower is available.

V. PRODUCT DEVELOPMENT AND EVALUATION

- A. **Objective:** To optimize the physical and subjective characteristics of tobacco produced in the NET process for inclusion in cigarette models.

- B. **Results:** To help determine the optimum physical and subjective characteristics of BLDET and DETA tobacco produced in the NET process for inclusion in cigarette models, seven expansion runs of C34, #10 Bright, and BLDET were completed. The C34 was expanded at low and high thermal treatment using cased and uncased tobacco. Eleven BLDET, four DETA, and two C34 cigarette models were made using the C34 and #10 Bright components and the BLDET blend. These models have been given to Leaf for subjective screening. These tests should be completed by the end of January.
- C. **Plans:** Pending the outcome of the subjective evaluation tests, more cigarette models will be made and analytical and physical testing will be done by CI.

RESEARCH

2057725194

PROJECT NUMBER : 0008
PROJECT TITLE : Computer Applications Division
WRITTEN BY : J. Palesis, J. Blankinship, M. Allred
PERIOD COVERED : December, 1991

I. Intelligent Process Control

- A. **Objective:** Use machine learning and fuzzy logic to develop an intelligent control system for the Blended Leaf dryer belt.
- B. **Results:** We have continued to develop the C program which implements a knowledge-based control strategy for the BL Dryer Belt. Using process interface software (Labtech Notebook and Real Time Access), we interfaced the C program running on a Personal Computer with the Belt Tracking System. On-line testing conducted during the Christmas shutdown, demonstrated that this program achieves real-time communication with the dryer belt at a control cycle rate of 1/10 of a second which is satisfactory. These on-line experiments have also revealed some deficiencies in the control rules which we are presently correcting in order to improve the performance of the expert control system.
- C. **Plans:** Continue on-line testing and fine-tuning of this deterministic version of the expert control system. When performance is satisfactory, create a "fuzzy" version of the expert system by converting the "crisp" numeric values used in the control rules to "fuzzy" concepts such as "positive-large", "positive-small", etc. Compare the performance of the "fuzzy" system with that of the deterministic system and evaluate the advantages of "fuzzy logic" (approximate reasoning) for solving complex non-linear control problems such as the BL dryer belt tracking problem.

II. Machine Learning: AIM and Cigarette Sales Prediction

- A. **Objective:** Apply machine learning to develop an empirical model for predicting yearly cigarette sales of full and generic brands.
- B. **Results:** Working jointly with Technology Assessment, we applied the AIM machine learning software to a set of consumer data and induced a predictive model for cigarette sales. The induced model is a polynomial network which predicts yearly sales of both full and generic brands based on the price of full brands and the "elasticity" of full brand price, i.e., the change in price from one year to the next. This model was tested with existing data and was found to perform as well as two regression models which were previously developed at PM.
- C. **Conclusions:** The application of machine learning to this problem demonstrates that machine learning tools such as AIM can substantially facilitate the development of empirical models. Whereas development of the two statistical regression models took trained statisticians several months to develop, the AIM model was developed in a matter of days and did not require any knowledge of statistical techniques.

- D. **Plans:** Continue to work with Technology Assessment to expand and refine the model. Arrange a training seminar for AIM.

III. Machine Learning: 1st-Class Tobacco Sheet Production Cost Modeling

- A. **Objective:** Apply machine learning to build an empirical model for estimating tobacco sheet production cost.
- B. **Results:** The 1st-Class machine learning software was applied to a set of data provided by Walt Nichols of Process Development to build an empirical model for estimating the production cost of different types of tobacco sheets based on production dust level, washed burley stem level, and sheet strength level. Using this model, the least costly combination of tobacco sheet production specifications was identified.
- C. **Plans:** Continue to work with Process Development to apply machine learning tools to other cigarette production and design problems.

IV. Neurocomputing

- A. **Objective:** Develop programming techniques for use of the HNC ANZA Plus Neurocomputer and Neurosoftware. Investigate potential applications of neurocomputing and related statistical techniques to R&D problems, and apply where appropriate.
- B. **Results:** For the Low Sidestream Visibility project, a report was completed which documents the preliminary results of a statistical analysis and pattern classification study of gas chromatographic data from the Sidestream Chamber for control and low sidestream visibility cigarettes. A variety of quantitative techniques were used, including inferential statistics and multivariate analysis, as well as pattern classification techniques such as Learning Vector Quantization. Notwithstanding the depth of this study, the results are preliminary in the sense that reliable conclusions cannot be drawn yet due to certain limitations in the experimental data. Nevertheless, the results may be suggestive of certain differences in sidestream smoke between control and low sidestream visibility cigarettes. Additional experimental and analytical studies are recommended to better understand the analytical and subjective differences between control and low sidestream visibility cigarettes. The principal results were: (1) A list of statistically significant differences between chromatographic patterns of sidestream smoke for the control and low sidestream visibility cigarettes. (2) A neural network classifier, trained using Learning Vector Quantization, which can be used to classify chromatographic patterns of sidestream smoke into control and low sidestream visibility cigarettes. (3) A Principal Components Analysis and an analysis of variance were performed to determine which component scores are significantly different between the control and low sidestream visibility models. The loading coefficients of these components can be used to characterize the chemical differences (and the underlying mechanism) between the control and low sidestream visibility sidestream smoke. Data was also statistically analyzed from new

2057725196

chromatographic analyses, performed by F. Hsu, of sidestream smoke for control and low sidestream visibility cigarettes. Both normalized (to unit total area) and unnormalized (raw) chromatographic patterns were used in the data analysis. For both the normalized and unnormalized patterns, analyses of variance were performed to identify which chromatographic peaks (compounds) are significantly different between the control and low sidestream visibility cigarettes. This list was then sorted in descending order by the absolute percentage difference between the mean peak areas to determine the significantly different peaks which were most different (percentage-wise) among the control and low sidestream visibility cigarettes. A computer program for the Duncan Multiple Range Test was also written to determine significant groupings between the various control and low sidestream visibility models. A principal components analysis was also performed; scores for the two most important components were graphed to show the separation between the classes.

- C. **Plans:** Complete the analysis of the chromatographic patterns to determine useful interpretations to guide further investigations. Prepare a memo summarizing the results of the study.
- D. **References:** Memo to R. Comes, **Preliminary Results of a Statistical Analysis and Pattern Classification Study of Gas Chromatograms of Sidestream Smoke For Control and Low Sidestream Visibility Cigarettes.**

V. CTSD Modernization

- A. **Objective:** Utilize "standard" PC or UNIX workstations to acquire data from CTSD lab instruments. The new systems will be more flexible and easier to maintain than the old prom-based data acquisition units built in-house.
- B. **Results:** The GC labs were modernized by installing HP 5890 GCs and utilizing the PC-based HP GC Chemstation product for collection and analysis of chromatographic data. Custom software was written to handle a barcode reader, create summary reports and exchange information with the host computer.

The Gas Phase lab was modernized by installing a Laser Precision FTIR instrument to analyze the gases in cigarette smoke. Custom software was written to communicate with the smoker controller, communicate with the FTIR instrument, generate reports, handle a barcode reader and to exchange information with the host computer.

The Physical Testing Robot is a new instrument that performs multiple physical tests on cigarettes and filters. Programs for the PC that interact with the robot were written by Development Engineering. Programs for the PC that exchange information with the PT Robot system, exchange information with the host computer, and interact with the operator were written by Computer Applications.

In the Smoking Lab, the manual weighing application station was implemented on a unix workstation. Communications with the Weighing Robot are still in development.

Other instruments for which data collection has been modernized include OV, Cigarette Length, and, most recently, Filter Length.

- C. **Plans:** Continue development in the Smoking Lab and Physical Testing Lab. Start development in the Materials Evaluation Lab. Provide support as needed.
- D. **References:** CAD Project Requests #3263, 3979, 4226, 4228, 4229

VI. Semiworks HP 1000 Replacement

- A. **Objective:** Migrate data collection and analysis from the Semiworks HP 1000 to the new VAX VMS system.
- B. **Results:** The programs for the VAX are developed. The new system is running in parallel with the old and differences are being logged and reviewed.
- C. **Plans:** Continue checkout until the end of January and then switch over to the new system.
- D. **References:** CAD Project Request #5230

PROJECT NUMBER : 1101
PROJECT TITLE : Entomological Research
PROJECT LEADER : D. L. Faustini
PERIOD COVERED : December, 1991

I. CIGARETTE BEETLE (CB) CONTROL PROGRAM

- A. Objectives: To determine (1) if resistance to methoprene is occurring in feral CB populations, (2) if physiological differences are present in laboratory vs field colonies regarding methoprene and (3) to investigate alternatives to conventional tobacco pesticides.

- B. Results: R,S-methoprene treated flue-cured tobacco (0-30 ppm) was made for use in screening bioassays. The F₂ feral larvae collected from Commerce Road warehouse #44 are now ready for methoprene screening bioassays (1).

The F₂ feral CB population collected from Kenbridge, Va. have started to emerge and are being transferred to tobacco.

Feral CB collected from Maury St. warehouse #28 have appeared to be infected with *Bacillus cereus* (2).

In collaboration with Project 6902 personnel, training was received in the use of the methoprene ELISA plate kit which included tobacco extract sample preparation (3).

Injections of 10 µl of freshly collected tobacco hornworm purge fluid resulted in mortality of 3rd and early 4th instar larvae. Injections of 50 µl purge fluid into early 5th instar larvae did not cause mortality (4).

- C. Plans: Set up a methoprene feeding bioassay using the F₂ larvae collected from Commerce Rd. warehouse #44. If any F₂ larvae from Maury St. warehouse #28 are available, they will also be screened.

D. References:

1. Minor, M. F. Notebook No. 9024, pp. 49-50.
2. Fletcher, L. W. and Thompson, J. V. (1972). A pathogenic strain of *Bacillus cereus* isolated from the cigarette beetle, *Lasioderma serricorne*. J. of Invert. Path. 20: 341-350.
3. Tenhet, S. W. Notebook No. 9091, pp. 32-34.
4. Coar, D. L. Notebook No. 9112, p. 60.

II. SERVICE TO OTHERS

- A. Objective: Provide technical services to areas outside R&D.
- B. Results: A visit was made to a PM International operation regarding CB customer complaints (1). As a follow-up to this situation, six cases of cigarettes were isolated from cold storage and inspected for CB activity (2).

A total of 28 insect-related domestic consumer complaints were reviewed during this report period (2).

CB efficacy tests were performed with the vacuum steam, probe-equipped conditioning unit at MZM - McKinney. Preliminary findings indicate that all four life-stages were killed during the 2-step conditioning cycle (3).

- C. Plans: Continue to assist with CB efficacy studies at McKinney and PM's 20th St. facilities; examine CB related customer complaints as requested by Product Audit personnel; and issue the annual update to the PM Infestation Control Manual.

D. REFERENCES

1. Faustini, D. L. Review of Product in Distribution - Japan. Memo to Krausse, S. C.; 1991 November 19.
2. Tenhet, S. W. Notebook No. 9091, pp. 32-34.
3. Tickle, M. H. Notebook No. 9124, pp. 53-54.

PROJECT NUMBER : 1720
PROJECT TITLE : Analytical Microscopy
PROJECT LEADER : V. L. Baliga
PERIOD COVERED : December, 1991

I. REDUCED SIDESTREAM/FILTRATION/PAPER TECHNOLOGY (Thompson)

- A. **Objective:** Examine the ultrastructure of selected cigarette papers, paper additives, and filtration materials in support of the paper technology project.
- B. **Results:** Four samples of Cellulon were examined that had been homogenized at different nozzle pressures and had been passed through the nozzle in a single or a multiple pass. The different homogenization procedures did not change the individual fiber widths which remained unchanged at 20-60nm, but the aggregate size of the fibers was increasingly smaller with increasing nozzle pressure. Fiber length could not be determined.¹
- C. **Reference:**

Thompson, L., "Examination of Cellulon Fibers," Memo to N. Gautam, December 12, 1991.

II. SUPPORT TO OPERATIONS and R&D (Baliga, Miser)

- A. **Objective:** To provide support for operations and R&D.
- B. **Results:**

Two samples of building materials from the Cabarrus complex were examined. One material consisted of a tar-based material with fiberglass cords aligned in one direction and spaced at uniform intervals. The second material was a heavy gauge plastic that was covered with sand and clay debris. In addition, a white honeycomb material similar to that of a solid foam was found on the surface of the second material.²

Carbon pellets from an unlabeled drum were determined to be coal-based carbon with no additional additives. This was compared to a known carbon which also was a coal-based carbon but contained Fe and Zn as additives.²

C. **References:**

1. Baliga, V. Miser, D., "Identification of Building Materials from Cabarrus," Memo to G. Vilcins, December 4, 1991.
2. PM Notebook #8911, p. 71.

PROJECT NUMBER : 1757
PROJECT TITLE : Analytical Specifications
PROJECT LEADER : D. Ingraham
WRITTEN BY : B. Baronian
PERIOD COVERED : December, 1991

FLAVOR AND ADHESIVES SPECIFICATIONS AND CERTIFICATION

- A. **Objective:** To develop analytical and sensory specifications for current, incoming flavor materials and to transmit specifications and methods for monitoring specifications to appropriate groups. To certify that PMI export flavor materials meet GFO, to issue a certification of analysis and to transfer methodology and certification to the Flavor Center. To develop specifications and analytical methods for adhesives used by PM USA.

B. **Results:**

Additional dispersion pigments have been analyzed for the item used in the blackening of the brand labeling. These additives have contributed unacceptable subjective notes to products being evaluated.

Three adhesives used in Project Bold are currently under evaluation.

Specifications for Tow Anchor adhesives have been completed and released and a status report on the Tow Anchor tank study was issued to Operation Services.

Fifteen test adhesives have been analyzed for R & D approval prior to factory use.

The determination of formaldehyde, acetaldehyde and boric acid content in adhesives was conducted by an HPLC method. Further refinement of the method is required to provide compositional confirmation.

The blended dry flavor program with its consolidation of two methods has been successfully completed. The blended dry favor specifications and methods package was completed and delivered to Technical Services.

A second set of RL samples was analyzed as a part of qualification test for a contract lab for RL Sheet Certification Program. Meetings were held with FTR to determine the format desired for RL Sheet Certification.

A collaborative effort with FTR is underway to investigate key marker components in flavors of interest.

Support to the Flavor Center continues as regards both flavor specification and export certification.

- C. **Plans:** Continue support to the Flavor Center on an as-needed basis. Continue analytical methods development and analysis of adhesives. Continue visits with key vendors on technical issues.

PROJECT NUMBER : 1761
PROJECT TITLE : Materials Evaluation and Infrared Investigations
PROJECT LEADER : G. Vilcins
PERIOD COVERED : December, 1991

I. INVESTIGATION OF HONG-KONG MARLBORO CASES

- A. **Objective:** To determine the identity of the contaminant.
- B. **Results:** Marlboro cases with cigarette cartons were submitted for the analysis of the cases, cigarette carton overwraps, cigarette packages, and the cigarettes to determine the identity of the oily stains found on these samples. Mass spectrometric, GC-MS, TC-MS, and FTIR analyses were performed on these samples. The major portion of the contaminant on the cases (#1924 and #1474) was a long chain hydrocarbon oil. The GC-MS analysis also picked up sesquiterpene type components, which may account for the spicy odor, in the stained cases, tarnished cigarette packs, and the cigarettes in these tarnished packs. The cases with no visible stains did not contain the hydrocarbon oil. The stained case from Richmond beige warehouse and a piece of a pallet from this warehouse contained a hydrocarbon oil similar, but not necessarily identical, to the Hong-Kong samples. However, in these samples no sesquiterpenes were found.
- C. **Plans:** Further experiments are planned with the hydraulic oil from the palletizer to check its composition and effect on the cigarettes with heating.
- D. **References:**
1. Magin, D., "Hong-Kong Cigarettes," memo to G. Vilcins, December 10, 1991.
 2. Vilcins, G., "Investigation of Hong-Kong Marlboro Cases," memo to D. Knudson, December 11, 1991.
 3. Sumpter, T., and Jensen, N., "Mass Spectral Analyses of Damaged Cases From Hong Kong," memo to G. Vilcins, December 4, 1991.

II. INFRARED ANALYSIS OF PM-WEB WITH CB-10 GUAR

- A. **Objective:** To investigate the possibility of measuring by infrared spectroscopy the amount of CB-10 guar applied to the web.
- B. **Results:** Samples of web with no guar have been compared to web material treated with 1%, 2%, and 3% guar. The samples were analyzed by measuring the infrared absorbance. Area integration was performed on selected absorbance bands of normal and subtracted spectra.
- C. **Conclusions:** Infrared spectra of the samples which had been treated with guar indicated an increase in the web thickness. Some areas of these spectra had general

absorbance band increases, but no clear pattern to these increases and application rates was evident at this time.

- D. **Plans:** Additional samples will be analyzed after attempts to reduce infrared energy scattering. New absorbance bands will be selected for area integrations to measure sample variability.

E. **Reference:**

Lyons-Hart, J., PM Notebook #7820, pp. 84-86.

III. **ADHESIVE SPECIFICATION**

- A. **Objective:** To characterize the various types of cigarette adhesives and identify the major components by infrared analysis.
- B. **Results:** Numerous adhesives were analyzed this month. The types of adhesives submitted were carton end, blank and innerframe, stamp, tow anchor, and promotional type adhesives.

C. **Reference:**

Griff, M., PM Notebook #9135, pp. 72-73.

IV. **MATERIALS EVALUATION**

- A. **Objective:** To identify the chemical composition of materials used in cigarette manufacturing.
- B. **Results:** Numerous chemical identifications were made of materials used in the manufacturing facilities. The results and the recommendations were entered into the Materials Evaluation data base and reported to Quality Assurance. Five different colored inks from Australia were analyzed and compared to the inks of similar colors used in Manufacturing Center. The analyses were made employing infrared and X-ray spectroscopic procedures.
- C. **Plans:** The materials evaluation program will be continued.

D. **References:**

1. Coleman, S., PM Notebook #9128, pp. 42-46.
2. Griff, M., PM Notebook #9135, pp. 65-71.

PROJECT NUMBER : 1762
PROJECT TITLE : Molecular Structure Determination
PROJECT LEADER : N. Jensen
PERIOD COVERED : December, 1991

ANALYTICAL INSTRUMENTATION SUPPORT AND DEVELOPMENTAL ACTIVITIES

A. **Objective:** During the past month project 1762 which includes analytical NMR, supercritical and tandem mass spectrometry labs and the analytical electronics shop was formed as a new project group. Assessment of present activities of these labs and development of new cooperative activities with other areas is in progress.

B. **Results:**

Activities in the supercritical lab included preparing reports for studies done in collaboration with T. Howell on extraction of fats from peanuts and coconut, installation of a new supercritical extractor, and preliminary planning of projects using supercritical extraction for preparation of samples containing the pesticide Aldicarb and for analysis of APA in cased filler.

The installation of a new solid state NMR is in progress in the NMR facility. Considerable NMR analytical support for the cast leaf project is in progress. Present studies are addressed towards following the fate of pectin in the process.

Mass spectrometry activities have included continued support for analysis of organic bromines in magnesite and associated papers, support for pesticide analysis including trace level analysis for Dimefox, support for analysis of reaction mixtures as well as purified compounds for CRD, preliminary investigation of direct measurement of nicotine from green tobacco leaves, and LC-MS analysis of PMT metabolites. Fundamental mass spectrometry research has focussed on development of the MS-MS-MS (MS^3) or three stage tandem experiment. This method has considerable potential for structural characterization of complex biological compounds especially a number of flavor materials. Plans have been developed with H. Nakatani for a preliminary peptide sequencing experiment and for the use of ^{18}O water in combination with digestion for determination of the C-terminus in peptides.

C. **Plans:** Results of these various support activities will be appropriately reported. In addition to the collaborative support projects mentioned above further new collaborations are expected. Fundamental studies regarding the utilization, extension of application, and development of new analytical capabilities with this analytical instrumentation will be pursued.

PROJECT NUMBER : 1902
PROJECT TITLE : Tobacco Microbiology
PROJECT LEADER : D. M. Teng
WRITTEN BY : J. B. Jones
PERIOD COVERED : December, 1991

I. TOBACCO MICROBIOLOGY

- A. Objective: To develop methods and to evaluate the microflora in tobacco materials.
- B. Results: Samples of PG that were judged unacceptable (1) by Flavor Center personnel were assayed for bacteria, mold, and yeast per the Project 1902 SOP (2). There was no microbial growth detected in any of the 5 samples that were evaluated. Results (3) will be communicated to the appropriate personnel.

At the request of R&D personnel, bacterial isolates were obtained from various samples of RCB sheet and the Siva and Asta processes. These isolates were identified as *B. pumilis* or *B. subtilis* on the Vitek® per the SOP (4,5). A memo will be issued on the results.

Samples of burley tobacco were microbially assayed per the SOP (2) and plated on Czapek Dox agar to isolate field molds, such as members of the genera *Alternaria*, *Cladosporium*, and *Rhizopus*, vs storage molds, such as members of the genera *Penicillium* and *Aspergillus* (6). Results are being analyzed.

C. References:

1. Chadick, D. Personal communication with J. Lewis; 1991 December 10.
2. Crockett, E. A. Special Report No. 86-055; 1986 February 20.
3. Chadick, D. Notebook No. 9044, p. 89.
4. Chadick, D. Special Report No. 89-001; 1989 January 5.
5. Chadick, D. Notebook No. 9044, pp. 84, 87-88.
6. Welty, L. and Lucas, G. B. Fungi Isolated from Flue-Cured Tobacco at Time of Sale and After Storage in Applied Microbiology; 1969 March, Vol. 17 #3, p. 360-365.

II. TOBACCO OPTIMUM MOISTURE STUDY

- A. Objective: To determine the moisture content of tobacco under which tobacco will not show visible mold growth.

2057725206

- B. Results:** The initial work on the correlation between Humidat® available water (Aw) with tobacco oven volatiles (OV), for strip tobacco (1), has been expanded (2). Initial values in bright, Oriental, and burley tobaccos covered only the normally expected range of tobacco OVs (~10 - 20%). The Humidat® readings for recent samples of tobacco moisture studies have been outside the range calibrated in the original memo (1). In order to extend the Humidat® range, DBC bright, DBC burley, and MT tobaccos were sprayed with different known amounts of water to increase their Aw levels. The tobaccos were allowed to equilibrate overnight in covered containers. Samples were taken simultaneously for immediate Humidat® analysis and OV analyses by ARD personnel. The results from both assays were correlated against each other using statistical packages available in RS1. It is recommended that 3 Humidat® readings be taken per sample and the average used to calculate the corresponding OV. The conversion equations for the 3 tobacco types have been established and a SOP is being reviewed (3,4).

Three grades of burley tobacco (B3X, B22, and B66) were obtained for the burley tobacco moisture study. The estimated starting date for the study is the week of January 6, 1992.

C. References:

1. Gaines, O. Standard Operating Procedure (SOP) for the HIC-2 (Humidat®). Memo to Dr. I. Uydess; 1989 April 10.
2. Thompson, N. Notebook No. 8779, pp. 156-158.
3. Crump, J. and Kiernan, B. O.V. Test for Strip Tobacco. Memo to J. Banyasz and B. Semp; 1988 March 22.
4. Teng, D., Thompson, N. and Weissbecker, L. Addendum to the SOP for the Humidat® (prepared, waiting approval).

III. NICOTINE BIOREMEDIATION

- A. Objective:** To facilitate/monitor the bioremediation of nicotine to simple compounds using indigenous microflora.
- B. Results:** A shake flask assay to study the removal of nicotine from Park 500 primary sludge (PS) was run (1). This study was conducted at 10°, 25°, and 37°C for 48 hours while shaking the loosely capped flasks at 100 RPM. Nicotine, dissolved oxygen, and pH analyses were performed on the above mentioned samples at 0, 2, 4, 6, 8, 12, 24, and 48 hours. Nicotine degradation occurred in the primary sludge after 12 hours in the 10° and 25°C flasks. Nicotine degradation occurred in the primary sludge after 48 hours in the 37°C flasks.

- C. Plans: The nicotine bioremediation of primary sludge will be scaled up to 4 liters in fermentors to include running primary sludge at 25°C at 100 and 250 RPM with/without aeration for a 7 day period.

D. Reference:

Jones, J. Notebook No. 8590, p. 172.

2057725208

PROJECT NUMBER : 1904
PROJECT TITLE : Tobacco Physiology and Biochemistry
PROJECT LEADER : D. J. Ayers/G. J. Patskan
WRITTEN BY : V. S. Malik
PERIOD COVERED : December, 1991

I. LOW NICOTINE STUDY

A. **Objective:** To investigate the biochemistry of the nicotine biosynthesis pathway and to use this information to generate tobacco plants with reduced nicotine levels.

B. **Results:** The roots from 32 hydroponically grown burley 21 tobacco plants were harvested and stored at -80°C (1,2,4). These plants were harvested 3 days after topping. The average weight of roots from this group of plants (group 32) was 159 grams compared to 216 grams (group 31) and 262 grams (group 30). Ammonium sulphate extracts were prepared from tobacco roots and further fractionated through the Phenyl Sepharose stage of purification. Various conditions to elute PMT activity from Phenyl Sepharose columns were investigated. However, none of these were found to be superior to the procedures currently in use (3). PMT-active peak and tail fractions were pooled, dialyzed and frozen at -80°C . PMT-active samples from a preparatory Phenyl Sepharose column were bound to a large DEAE Sepharose column. The PMT was eluted off the DEAE Sepharose column and adsorbed to the AHS column. The PMT was eluted off the AHS column with 1.5 M NaCl and further concentrated using a Centricon 30s. Aliquots of the concentrated PMT preparation called Batch 7 have been frozen (4). Conditions for the use of HPLC for purifying PMT were explored (5). The remaining Batch 6 samples have been processed by HPLC and GPC (6).

Construction of chimeric plasmids which could express tobacco root cDNA sequences in antisense orientation continued. Several cDNA fragments that are overly expressed in tobacco roots but lack a poly A region were successfully inserted in antisense orientation in the plant vector pVSTI (7,8). The DNA of these recombinant plasmids is being prepared for use in transforming tobacco. A total of 15 control transgenic plants transformed with pBI121 were generated. Of these 10 were transferred to the greenhouse for acclimatization. Additional plantlets transformed with the antisense constructs p17.2A and p12A have been generated and are being prepared for graduation to the greenhouse. Shoots from transformants involving 10 additional antisense constructs have been transferred to rooting medium for regeneration of transgenic tobacco burley-21 plants (9). Exploration of the methodology that utilizes antibodies for characterization of proteins and isolation of genes was begun using PET antibody (10).

C. **Plans:** Generate antibodies against 60 kD protein. Use PCR to isolate DNA sequences related to the putative PMT. Generate purified PMT fraction of Batch 1 and generate additional amino acids sequences of putative PMT protein.

D. References:

1. Lyle, J. Notebook No. 9077, p. 67.
2. Turner, D. Notebook No. 9094, p. 190.
3. Steele, M. Notebook No. 9068, p. 106.
4. Davies, S. Notebook No. 9103, p. 150.
5. Yu, T. Notebook No. 9002, p. 165.
6. Nakatani, H. Notebook No. 8384, p. 192.
7. Wahab, S. Notebook No. 9151, pp. 27-28.
8. Malik, V. Notebook No. 8974, p. 154.
9. Shulleeta, M. Notebook No. 9132, p. 30.
10. Vaughan, B. Notebook No. 8948, pp. 163-164.

PROJECT NUMBER : 2500
PROJECT TITLE : Inorganic Synthesis
PROJECT LEADER : K. F. Podraza
PERIOD COVERED : December, 1991

I. INORGANICS AS NOVEL TOBACCO MATERIALS ADDITIVES

(Fournier, Kallianos, Paine, Podraza)

A. Objective: To develop inorganic materials for novel applications for reduced sidestream smoke, for burn-rate modification, enhanced subjectives in cigarettes and for required properties in novel smoking articles.

B. Results and Plans:

i. **Magnesite.** The magnesite sample from the 10-gallon sweetening run conducted at Pressure Chemical Company was received. TG/DTA showed no signs of $\text{Mg}(\text{OH})_2$, indicating 99+% conversion to magnesite. The mean particle size was determined to be about 12 μm , similar to a magnesite sample prepared at PM but with a narrower particle size distribution. Analyses for Fe, Ni, and Cr showed no increase in the levels observed in the $\text{Mg}(\text{OH})_2$ starting material (Fe 86 ppm, Ni and Cr < 5 ppm). The sample exhibited good handsheet-making properties and acceptable brightness. Pressure Chemical Company has been instructed to begin production of the 200 pounds of material.

ii. **Aqueous Non-Sol-Gel.** Subjective evaluations were completed on two cigarette models fabricated from handsheets containing mag carbonates prepared by two alternative aqueous non-sol-gel routes, using starting materials and preparative conditions suitable for large-scale syntheses. Both models were judged to be subjectively acceptable. Based on subjectives and ease of preparation, the 50:50 (w/w) composition has been selected for scale-up synthesis. Reheis magnesium hydroxide paste is the sole starting material in this two-step, two-pot reaction. Currently, a technical package is being compiled with Dr. W. B. Edwards to allow scale-up.

iii. **Aqueous Sol-Gel.** The aqueous sol-gel procedure involves mixing a magnesium bicarbonate solution, prepared by reacting hydromagnesite with carbon dioxide, with a solution of magnesium acetate and heating this mixture to 50°C followed by addition of potassium hydroxide. TGA and X-ray evaluation of the products indicated that they consist of hydromagnesite and magnesium hydroxide. SEM evaluation, however, showed only a single phase, characteristic of hydromagnesite rosettes with no clear evidence of the magnesium hydroxide. The particle size of magnesium hydroxide in these mixtures, using half-peak width from x-ray diffraction data, were approximately 0.01 μm or 100 angstroms. Electron diffraction data indicated a particle size for the magnesium hydroxide microcrystal to be smaller than 0.02 μm or 200 angstroms. Additionally, diffraction patterns of the rosettes confirmed the presence of hydromagnesite and locate the magnesium hydroxide as a coating on some of the rosettes as well as entrained only in the outermost portions of the rosettes.

iv. Calcium Carbonate. As the outcome of a planning meeting held on the subject of calcium carbonate fillers, a wide range of commercial ground limestones and precipitated calcium carbonates were made into handsheets (33% filler level, 47.5 gram/square meter basis weight) and sized with 10.5% potassium dihydrogen phosphate or 8% potassium succinate. The phosphate-sized models behaved similarly for all of the ground limestones or PCC's except one: static burn times were unacceptably long, or else the models refused to burn. By contrast, the model containing Multiflex MM stood out in having a very fast SBT, even when sized with potassium dihydrogen phosphate. With potassium succinate sizing, the two Micro-Whites models gave a similar sidestream reduction, 41 to 49%, yet had significantly different porosities (9.2 versus 5.6).

Additional calcites supplied as slurries were filtered and dried, and are currently being examined photomicrographically.

Machine-made papers are being prepared at the University of Maine using four different calcites as filler. The four calcites, Albacra 5970, Microna 3, Multiflex MM, and Micro-White 25, were chosen because of their similar particle size ($\sim 5 \mu$, Malvern Analyzer), different morphology, and commercial availability.

v. Spray Drying. Smoking results were received for two sets of cigarettes made with papers containing a total filler level of 30% comprising 10% MM calcium carbonate/20% hydromagnesite/magnesium hydroxide. The hydromagnesite/magnesium hydroxide sample was generated from the reaction of a magnesium acetate solution with a solution containing potassium carbonate and potassium hydroxide and was used in the filtered paste form or was spray dried before use. The model using the filtered paste form of the hydromagnesite/magnesium hydroxide sample resulted in a 78% reduction in sidestream smoke (SBT 10.6 min.). In the case of the spray dried sample, a 70% reduction in visible sidestream smoke was obtained (SBT 8.8 min.). Both sets of cigarettes will be subjectively evaluated.

vi. Mechanistic Studies. A paper sample (P1YA-#1) was examined for porosity before and after impregnation with organic liquids (e.g., quinoline, dimethyl sulfoxide, and nicotine) specifically chosen for minimally evaporation during the course of the porosity measurements. All three reduced the permeability by 40-50% at a weight add-on in the range of 30-40%. Quinoline was examined on the same strip of paper at several increasing levels of incorporation: permeability fell monotonically with increase in weight add-on of the liquid. In a functioning cigarette, both the high concentrations of water vapor, and the thermal gradient, are expected to modify this effect.

vii. Burn Rate Modification. Magnesium ethyl carbonate was obtained as a 53% (w/w) solution in ethanol by the novel approach of dissolving magnesium metal turnings in anhydrous ethanol through which carbon dioxide was passed continuously. Carbon-13 NMR of the reaction mixture as obtained showed ethyl carbonate resonances in addition to the solvent peaks. The material adhered nicely to paper, forming an impregnating film. Exposed to moist air, the material began to gel at once, eventually forming a glass that crumbled to a powder. Pyrolysis GC/MS

revealed that ethanol or ethyl carbonate residues are present in this solid. FT/IR of the solid showed the presence of carbonate peaks, but not at positions corresponding to any of the usual magnesium carbonate phases previously encountered. Preliminary results indicate that magnesium ethyl carbonate may be useful as a sizing agent for burn rate modification.

Structures of Substrates.

calcite, calcium carbonate, $[\text{CaCO}_3]$

hydromagnesite, $[\text{Mg}_5(\text{CO}_3)_4(\text{OH})_2 \cdot 4\text{H}_2\text{O}]$

magnesite, $[\text{MgCO}_3]$

magnesium hydroxide, brucite, $[\text{Mg}(\text{OH})_2]$

II. ENVIRONMENTAL STUDIES (Howe, Podraza)

A. Objective: To apply chemical expertise to the identification and development of techniques which reduce environmental pollution.

B. Results and Plans:

i. **Resin Regeneration.** Polymeric resins such as XAD-4 (Rohm and Haas), adsorb nicotine from water and air streams. Removal of the nicotine from the XAD-4 resin, to regenerate the resin, has been investigated with 95% ethanol. A UV spectrophotometer has been installed in our lab (with the assistance of D. Watson and S. Yang) to perform the nicotine analyses. The XAD-4 resin was initially loaded with 1% or 5% nicotine and allowed to air dry. The 95% ethanol was then passed through the dry resin. Three bed volumes of ethanol removed approximately 87% of the nicotine. Additional studies are in progress evaluating other solvent systems.

ii. **TKN.** Discussions with our consultant regarding the TKN method revealed a number of areas which should be examined at PM. Experiments are being designed to determine the influence of temperature on the TKN method, nitrate levels as a potential interference, total nitrogen analysis by combustion, and organic nitrogen levels. Methods to determine what contributes to refractory nitrogen and techniques to remove the refractory nitrogen are being explored.

iii. **TCLP Analysis of RLPF Waste.** Two waste samples from the RLPF have been extracted according to the TCLP protocol. The analysis of this extract for the TCLP specific insecticides and herbicides are expected to be completed by the first of the year. Additional samples of bright tobacco stemmery waste was obtained for a more detailed study. Currently, samples of burley tobacco stemmery waste is being collected.

III. MISCELLANEOUS (Howe)**Menthyl isopropenyl Carbonate (MIC)**

The synthetic procedure to prepare menthyl isopropenyl carbonate, involved reacting the sodium enolate of acetone with menthyl chloroformate, was conducted several times. The yield of MIC was $\leq 30\%$.

PROJECT NUMBER : 2501
PROJECT TITLE : Smoke Chemistry
PROJECT LEADER : D. D. McRae
PERIOD COVERED : December, 1991

I. SIDESTREAM SMOKE CHAMBER

- A. **Objective:** Operate an environmentally controlled chamber to measure selected components of sidestream smoke.
- B. **Results:** Sidestream smoke collection runs using X8D9XO cigarettes have been completed. These runs were performed at the request of Biochemical Research.

Chamber runs for the magnesite experimental series have also been completed. All analytical measurements except for nicotine have been performed.

The chamber has been shut down for maintenance during the week of December 16th and for the general Tower closing over the Holidays.

- C. **Plans:** Finish analytical measurements and report the results for the magnesite models. Complete chamber maintenance. Perform further chamber runs to test the new aerosol particle size measuring equipment.

II. SIDESTREAM SMOKE: CHEMICAL PROPERTIES

- A. **Objective:** Conduct studies on sidestream smoke including: development of methods for collection and analysis of sidestream semivolatiles and gas phase; visibility determinations; analysis of selected materials relating to sidestream odor and irritation; development of proprietary products.
- B. **Results:** Headspace gc/ms analyses of the 30-cigarette runs of X8D1WG (control) and X8D1WJ (70% sidestream reduction) were completed. Because larger amounts of material were collected in the 30-cigarette runs, more gc peaks could be detected and a new analysis procedure was developed. About 450 peaks were detected out of which 230 were tentatively identified. This data was transferred to J. Blankenship for statistical analysis. The statistical analysis identified compounds that are present in the sidestream smoke of each cigarette type at significantly different levels.

The gc sulfur detector has been transferred to F. Hsu for planned experiments in Analytical.

- C. **Plans:** Headspace gc/ms data for the magnesite models will be submitted for statistical analysis. Plans are being made to collect sidestream gas phase samples from the prototype CORESTA smoking machine. These samples will be compared to the samples collected from the chamber.

III. SIDESTREAM SMOKE: PHYSICAL PROPERTIES

- A. **Objective:** Conduct studies on sidestream smoke including: prototype CORESTA smoking runs; particle size and visibility determinations; mechanistic studies; infrared camera and video observations.
- B. **Results:** Video taped images of the magnesite models and their control have been reviewed. The magnesite models have perforated paper and sidestream smoke is clearly seen to exit through the perforation holes. However, the mass of sidestream smoke emerging from the perforations is probably quite small and is not normally visible to the casual observer. The smoldering magnesite models look very similar to previous low sidestream cigarette models. However, we have seen some unusual events that will require more study to determine their significance.

Chamber runs to measure the aerosol properties of the magnesite series have been made. Several more runs will be needed to complete the data set. These runs will also be used to provide data to compare the results from the new and old particle size measuring instruments.

CO and CO₂ monitors are being tested in preparation for their use with the new prototype CORESTA smoking machine.

- C. **Plans:** Prototype CORESTA smoking runs will be performed on an as needed basis. Construction of the second generation machine will be monitored. Analysis of IR images will begin once the image processor board is reinstalled. Aerosol chamber runs will be completed.

IV. MISCELLANEOUS

1. Pyrolysis gc/ms analyses have been performed on a variety of compounds.
2. Samples from the isolation of compounds from carbon-14 radiolabelled tobacco were analyzed with the Harvey oxidizer to determine specific activities.
3. Additional nicotine analyses were performed in support of work to determine the feasibility of using resins to trap nicotine from waste streams.

PROJECT NUMBER : 2520
PROJECT TITLE : Organic Synthesis
PROJECT LEADER : Y. Houminer
PERIOD COVERED : December, 1991

I. FLAVOR RELEASE TECHNOLOGY

- A. **Objective:** To investigate the synthesis, pyrolysis and performance of various flavor release systems for use in new or improved products.
- B. **Results:** In order to satisfy a request by Flavor Technology for 100 gm of PolyMIC polymerized with the same initiator used for the preparation of PolyItaconate and PolyAllyl, we have begun to investigate the polymerization of MIC with t-amyl peroxy-2-ethylhexanoate. A sample of the monomer was purified by dry column chromatography on silica gel and a small scale polymerization was conducted for 40 hrs at 80°C to yield 62.7% of the pure polymer.

We are currently developing a precipitation procedure for polyMIC which avoids methylene chloride and methanol used in previous polyMIC preparations.

II. FILTER MATERIALS AND PAPER TECHNOLOGY SUPPORT

- A. **Objective:** To find outside suppliers for large quantities of new filter materials and inorganic paper additives.
- B. **Results:** Pressure Chemical has completed all the necessary changes and conditioning of their 10 gallon reactor for the hydrothermal preparation of magnesite. Magnesite from the first 10 gallon run has been received and is >99+% pure. Target metals are within specification. The material appears to have a smaller particle size than that prepared in the 1 gallon reactor. Work is in progress to determine if this is acceptable.

A joint confidentiality agreement and joint development agreement, covering fibrids, has been signed by PM and sent to Courtaulds for signature.

Initial work has been completed by K. Newman on the experimental sheet materials received from Courtaulds. The 70/30 and 50/50 CA/Polypropylene mixtures show promise in preliminary filter making trials.

III. CAST LEAF

- A. **Objective:** To study the mechanism of binder-tobacco interaction and use the data to improve both the process and the sheet properties.
- B. **Results:** Preliminary SEM analyses of different handmade cast leaf sheets have been completed. The results show that the uniformity of the sheet is higher when the tobacco feedstock particle is smaller. Higher quar gum content also produced better sheet. Optical microscopy showed increased number of pin holes in sheets obtained

from large tobacco particles as compared to smaller tobacco particles. The above results are in agreement with the physical measurements obtained for these sheets.

The amount of pectin released in the RCB/NBL process is being quantified. Laboratory as well as pilot plant samples are being analyzed by NMR.

PROJECT NUMBER : 2526
PROJECT TITLE : Greenhouse Operations
PROJECT LEADER : R. T. Bass
PERIOD COVERED : December, 1991

I. GREENHOUSE STUDIES AND RESEARCH ACTIVITIES

(R. Bass, G. Newell, G. West)

- A. **Objective:** Maintain the R&D Greenhouses, conduct plant research studies, provide greenhouse-grown tobacco materials for support of other R&D programs, and provide requested assistance for special projects.
- B. **Results:** The regular Greenhouse plant production activities have been completed on schedule, including the preparation of 220 gallons of Peters 5-11-26 nutrient solution, five insecticide applications, the performance of all cultural practices, and the seeding and transplanting of K-326 and Speight G-28.

The regular Greenhouse operations and maintenance tasks have been done as scheduled including the calibration of the heating system controls, the painting of the small headhouse interior and the lighting system support frame, and shutting down the Arctic cooling fan for the winter season.

The harvest operations for the Hydroponic Group 32 plants were completed. The fresh root material from the 52 plants of Burley 21 was provided for processing. Two plants were used for yield determinations and oven dried leaves will provide samples for analyses.

The ^{14}C -tobacco leaf material from Chamber Run No. 15 was taken through an extended oven curing procedure to minimize the chlorophyll content and hopefully enhance the content of other compounds of interest. A total dry weight of 256 gms. was provided to Dr. R. Izac for processing. After curing, leaf tissue samples were taken and specific activity determinations were made as follows:

<u>Sample</u>	<u>Specific Activity $\mu\text{Ci/gm.}$</u>
Top Leaves	271.4
Middle Leaves	253.3
Bottom Leaves	185.1
Overall Average	236.6

As requested by and in cooperation with the BCR Modified Alkaloid Tobacco Program, a group of tobacco plantlets have been set up on the Greenhouse bench for a hardening off process, to then be grown to maturity. We will be working with BCR to expand our efforts in this area in the future. Additional supplies and equipment will be obtained to facilitate this increased effort.

- C. **Plans:** Greenhouse operations and maintenance will be continued along with all requested support activities.

D. References:

1. G. Newell, N.B. No. 8762
2. G. West, N.B. No. 8559
3. R. Bass, N.B. No. 8999

II. COOPERATIVE TOBACCO VARIETY EVALUATION PROGRAM

(R. Bass, G. Newell and G. West)

- A. Objective:** To participate in the cooperative tobacco industry program in order to assure that the quality of all new tobacco varieties meets or exceeds all requirements as defined by minimum standards program.
- B. Results:** The total alkaloid and sugar analyses for the 1991 S.C. MH-30 Sucker Control Test have been completed and the results have been reported to D. Gooden at the Pee Dee Research Station and F. Yelverton at N.C.S.U.

The smoke flavor panel evaluation for the 1991 Flue-Cured Tobacco Regional Farm Test entries has been completed. The panel judged six of the ten entries in the test to be unacceptable in smoke flavor characteristics as compared to the control varieties.

The 1991 annual meeting of the Flue-Cured Tobacco Cooperative Variety Evaluation Committee was attended in Raleigh, N.C. on 12/10/91. After thorough evaluation of all agronomic, chemical, physical and smoke flavor characteristics for all entries, decisions were made as to their acceptability based on the minimum standards of the industry. For the 1991 Regional Small Plot Test, seventeen of the twenty three entries met the standards requirements and are eligible for advancement to the 1992 Regional Farm Test, seven of the ten entries met the minimum standards requirements and are eligible to be released as new tobacco varieties in 1992. Their release is optional with their sponsor. These seven entries are RG-11, NC8053 USDA, Reams 126, RG-13, Speight G-111, NCTG-52 and Reams 44. Mr. Reams announced his plans to release Reams 126 as Reams 126, and Reams 44 as Reams 744 for the 1993 season.

The following five new flue-cured tobacco varieties have been released for the 1992 season: NC 729, RG-8, RG-22, Speight G-117 and Reams 713. All of these have been through the variety testing program in previous years.

The Combined Cooperative Burley Tobacco Variety Evaluation Display will be held on 12/17/91 at Greeneville, Tennessee.

- C. Plans:** To continue to participate in the Cooperative Tobacco Variety Evaluation Program.

D. References:

1. G. Newell, N.B. No. 9090
2. G. West, N.B. No. 8559
3. R. Bass, N.B. No. 8999

PROJECT NUMBER : 2527
PROJECT TITLE : Radiochemical Services
PROJECT LEADER : A. F. Frisch
PERIOD COVERED : December, 1991

I. RADIATION SAFETY

- A. **Objective:** Provide support to all isotope users and monitor areas designated for tracer application studies.
- B. **Results:** The tobacco from chamber run number 15 was oven cured in early December. A lower starting temperature for the process was employed to see if this approach would reduce the green color of the leaf during the curing cycle. A total of 256 grams of dried leaf was obtained from the curing process. Samples of top, middle and bottom leaf were obtained for activity determinations using the Harvey Oxidizer. Results reported by B. Francis of Project 2501 show the average activity per gram of dried leaf to be 271.4 micro-Curies/gram for the top, 253.3 micro-Curies/gram for the middle, and 185.1 micro-Curies/gram for the bottom.

The residue from the extraction procedure of run number 14 has been examined for activity levels. Results indicate a value of 152 micro-Curies/gram. This value will be used to estimate the waste activity level for final burial.

The isolation of the C-14 solanesol from run number 15 has proceeded without difficulty. Final purification is complete and all that remains is to establish purity level and C-14 activity.

Dosimetry results for October are now complete for both isotope and X-ray film badge holders. All exposure data is within acceptable limits.

II. MISCELLANEOUS

Meetings were held with the Analytical Research Division and all contract cleaning personnel on December 6, 1991 in order to discuss the results of the NRC inspection of last September.

PROJECT NUMBER : 2705
PROJECT TITLE : Tomorrow
PROJECT LEADER : R. W. Dwyer
PERIOD COVERED : December, 1991

IGNITION CHARACTERISTICS OF CIGARETTES

- A. **Objective:** Develop tests to assess the ignition propensities of cigarettes.
- B. **Results:** We are extending the CORESTA-III tests to include the effects of substrate geometry. The earlier tests involved a specific fabric and foam combination in a flat configuration. In this latest round, we are examining the same cigarettes and substrates in an L-shaped configuration where the smoldering cigarette is placed in the crevice. A chamber has been built with which we can control and alter the flow rate of air across the smoldering cigarette. This testing is being performed in the labs of all of the Joint Venture members. Preliminary tests have been performed with materials on-hand, and no appreciable differences between these results and the earlier results on flat configurations have been noted. Additional tests will be performed when the treated fabrics prepared by Celanese are received.

A series of cigarettes has been fabricated in which the rod circumferences vary from 17 to 27 mm. These cigarettes will be included in the crevice tests.

A mathematical model of the substrate is being developed and tested with the data collected from earlier thermal-properties and ignition tests. The model requires a thermal distribution from a cigarette as input and calculates the temperature distribution on the fabric surface and the oxygen concentration at the fabric surface. The model includes the thermal characteristics of the substrates and accounts for evaporation from and pyrolysis of the surface.

- C. **Plans:** We shall measure the heat flux of freely smoldering cigarettes and cigarettes smoldering on fabric/foam substrates as functions of air flow. We are also measuring the linear and mass burn rates of cigarettes during crevice testing and monitoring the effects of substrate geometry on the coal lengths of smoldering cigarettes.

PROJECT NUMBER : 2706
PROJECT TITLE : Tobacco Processing Principles
PROJECT LEADER : T. M. Howell
WRITTEN BY : D. A. Lowitz
PERIOD COVERED : December, 1991

I. CAST SHEET DRYING MODEL

- A. **Objective:** Develop a computer based model that will mathematically describe the drying of cast sheet.
- B. **Results:** Our recently developed drying model was used in a computer simulation of the BL Plant operation at 330 fpm. Available data were used for the BL significant parameters such as equilibrium absorption, density, specific heat, and thermal conductivity. It was found that higher temperatures in Zone 4 to Zone 9 have a significant effect on the final OV level. New drying conditions were suggested for the BL Plant to achieve a 350 fpm throughput.
- C. **Plans:** The drying operation will be tested at 350 fpm using the model generated operating conditions. More accurate determinations of cast sheet properties will be made by performing a series of laboratory experiments and pilot drying tests.

II. VIBRATION ANALYSIS

- A. **Objective:** Develop vibration analysis as a tool for predicting and diagnosing machine health and as a design aid for improving machine performance.
- B. **Results:** Accelerometers have been installed on all SE spider assemblies and Protos 100 VE fans in MC Bay 1. A training program for Bay 1 personnel on data acquisition was initiated. Details of the accelerometer mounting procedures are being documented.
- C. **Plans:** (a) Complete the training of Bay 1 personnel on the accelerometer measurement procedures. (b) Fabricate laboratory test equipment to permit developing additional vibration analysis diagnostic techniques.

III. OFF-LINE INSPECTION

- A. **Objective:** Develop and implement systems for the inspection of incoming materials.
- B. **Results:** The motion control and material handling system under construction by PS & T has been developed to the stage where PS & T can sequence blanks through the system. The user interface software for the QA two-color inspection system has been developed to the stage that permits the grabbing and storing of master images.
- C. **Plans:** Complete the algorithm and user interface software for the system.

IV. NEW PRIMARY PROCESSING

- A. **Objective:** Evaluate the feasibility of applying microwave or radiowave (RF) heating to tobacco prior to cutting.
- B. **Results:** FDA and FCC supplied the current regulations on RF dielectric heating relevant to human safety and the avoidance of communications interference.

A sequence of RF heating tests were done on Bright, Burley, and Oriental leaf at PSC, Inc., of Cleveland, Ohio. The moisture content of the leaf ranged from 16 to 21% OV. The target temperature was 1400F. Depending on test conditions heating time ranged from 50 sec. to 3.5 min. to reach 1400F. The order of heating time according to leaf type was Bright> Oriental>Burley; and for moisture content, 16% OV> 18% OV>21% OV. In some cases fire occurred in the stem portion. In one case arcing was observed. However, it is possible that these problems can be eliminated by choice of suitable operating conditions of RF frequency, plate voltage, bed height, and leaf dielectric properties.

- C. **Plans:** Determine the dielectric properties of the leaf used in the study as a function of frequency and temperature. Develop techniques for eliminating stem fire and plate arcing.

V. PRINT WEB INSPECTION

- A. **Objective:** Develop a system for the global inspection of print web on the printing press.
- B. **Results:** A press at Fergusson & Sons (FS) was identified as suitable for testing the PM image grabbing subsystem. A mounting system for the PM cameras and lights was designed and is under construction. Arrangements were made with FS and Futec to test the Futec display system at the same FS press. A lease arrangement was made with Futec; their "evaluation" system will be here January 9, 1992. A test protocol for Futec was developed and has been approved by FS. A Futec mounting system is being designed.

Futec is the only commercial system currently available for 100% high speed print web inspection. If it can detect a large portion of the more blatant intermittent defects, it would provide good progress toward achieving savings in manufacturing. The PM system is designed as a higher resolution system with better capability for meeting PM Operations specifications.

- C. **Plans:** Install and test the Futec optical inspection system at Fergusson and Sons and independently test the image grabbing part of the PM system.

PROJECT NUMBER : 6502
PROJECT TITLE : Process Monitoring and Smoke Investigations
PROJECT LEADER : R. W. Kanipe
PERIOD COVERED : December, 1991

I. SIDESTREAM SMOKE

- A. **Objective:** To characterize the compositional differences of sidestream gas phase of reduced visibility cigarettes with KH_2PO_4 versus control cigarettes.
- B. **Results:** The glass enclosure of Jenkins' design was used to generate fresh and concentrated sidestream smoke from the reduced visibility model cigarettes: control (X8D1WG), 40% (X8D1WK), and 70% (X8D1WJ). The 40% and 70% reduction models contained 4.6% and 8.4% potassium phosphate in the paper, respectively. The gaseous portion, isolated by using a Cambridge pad, was introduced into a gas chromatograph by combining a dual-loop sampling valve and a cryofocusing injector. The sidestream smoke was sampled discreetly throughout the static burning of the cigarette. Sixty analyses (15 replicates/sample) were completed, and 150 peaks (compounds)/chromatogram were selected for chemometric analysis. The data were analyzed by analysis of variance (ANOVA) and principal component analysis (PCA) to reveal the differences and underlying pattern. Both the raw and normalized peak areas were used in the data analyses.
- C. **Conclusions:** With the raw peak areas, almost all of the peaks were found to be significantly different and therefore, there was no immediate reduction of variables (peaks). Examination of the principal component scores revealed that there were underlying groups of different chemical composition. The cluster of 70% model cigarettes, located at the extreme region of principal component 1 axis (PC1), indicated that they corresponded clearly to a lower delivery of methyl isopropyl ketone, 1-undecene, n-nonanol, acetonitrile, 2-methylpyridine, 2-butylfuran, benzene, and others to the sidestream smoke than the 40% and the control models. The clusters of 40% and control cigarettes, located adjacent to each other along principal component 2 axis (PC2), delivered very similar amounts of acrolein, benzonitrile, 2-methylcyclopentanone, furan, 2-ethylfuran, and other compounds. Removing the differences in the amount of sidestream smoke by normalizing the raw data, the PCA still indicated the existence of three separate groups with different lists of compounds.

II. QUALITATIVE ANALYSIS OF ADHESIVES USING FTIR SPECTROSCOPY

- A. **Objective:** To develop a rapid qualitative procedure for screening incoming adhesive samples at Cabarrus for gross contaminants.

2057725226

B. Status:

Efforts were made to obtain containers suitable for the headspace portion of the analysis to eliminate the need for a syringe needle in sampling. Individuals at CEM were contacted regarding the use of a microwave digestion vessel. These vessels were determined to be adequate with some minor modifications to be made in the lid covers. A valve will be added to the digestion vessel to facilitate sample transfer into the FTIR gas cell.

The FTIR instrument was installed at Cabarrus on November 6 by Will Lewis and Mark Allred. Demonstrations of the procedure were given to six QA employees. The procedure performed well and received favorable comments. Mark Atkins, our contact at Cabarrus, was given documentation for the automated procedure.

Mark Atkins is finalizing the necessary laboratory modifications required to purge the FTIR with clean, dry air. The R&D machine shop is modifying the CEM microwave digestion vessels for headspace analysis.

- C. Plans:** Plans are 1) to return to Cabarrus in December to evaluate the performance of the instrument to date, 2) to receive feedback from Cabarrus personnel on the procedure, 3) to evaluate the modified vessels. Mark Allred will continue to assist with software modifications and improvements, especially in the area of headspace contaminant evaluation.

D. Reference:

Lewis, W. Jr., PM Notebook #9149 p. 1.

III. WAREHOUSE MOISTURE STUDY

- A. Objective:** To monitor moisture changes of tobacco stored in PM80 boxes and to relate these changes to shifts in equilibrium within the boxes or moisture penetration from outside the boxes.
- B. Results:** Relative humidity and temperature probes (resistance) were installed in two positions of eighteen PM80 boxes. Readings from these instruments have been taken weekly. An additional eighteen recordings per box of moisture using radio frequency and dielectric probes were made. The total number of measurements was about 550 per week. Humidity data from the 3 week sampling suggested that the phase modulation (pm) of the dielectrometer was too unstable to be of practical value and therefore will not be recorded weekly. There appeared to be a correlation between the relative humidity and the moisture measurements.
- C. Plans:** Plans are 1) to continue recording data from these devices weekly, 2) to determine a suitable format to enter the data for statistical evaluation, 3) to monitor and "interpret" the results continuously.

2057725227

PROJECT NUMBER : 6503
PROJECT TITLE : Chemical Analysis
PROJECT LEADER : B. Handy
WRITTEN BY : W. Ryan
PERIOD COVERED : December, 1991

I. METHOPRENE (KABAT)

- A. Objective: Support engineering efforts to monitor uniformity of Kabat,, application in contract stemmeries.
- B. Results: Methoprene and OV analyses on Dryer Exit and Hogshead Core samples from Qualification Tests at Austin-Carolina (Line #1) and Standard Commercial stemmeries have been completed.
- C. Plans: Continue support as needed.

II. PROJECT TOMORROW

- A. Objective: To determine the level of potassium in fabric samples treated with potassium succinate.
- B. Results: Samples from two batches of treated fabrics were prepared by low pressure closed-vessel microwave digestion. The digested samples were analyzed for potassium by flame atomic absorption spectrometry. This one-step sample digestion technique is a significant improvement over the CORESTA sample preparation procedure used for previous fabric analyses. Use of this shorter digestion procedure and the more sensitive flame AA readout allowed analytical results to be reported in a more timely fashion.
- C. Plans: To continue to analyze samples as needed. Use XRF to determine the uniformity of potassium succinate application on the fabric.

III. TRIM SINGLE WRAP

- A. Objective: To provide support to the QE Microstructure Laboratory for the quality assurance testing of Trim Single Wrap paper.
- B. Results: One-half of the current shipment of Trim Single Wrap paper was analyzed by alternative colorimetric and titrimetric methods to verify the XRF results for phosphorus and calcium obtained by the QE Microstructure Laboratory.
- C. Plans: To continue to provide technical assistance to the QE Microstructure Laboratory as needed. To gradually reduce the number of samples analyzed by wet chemical methods for verification.

PROJECT NUMBER : 6505
PROJECT TITLE : Special Investigations
PROJECT LEADER : S. Yang
PERIOD COVERED : December, 1991

SUPPORT TO OPERATIONS AND R&D

A. Objective: To provide analytical support to R&D and operations personnel and projects.

B. Results:

Adhesive solutions and cigarette samples with a-hexyl cinnamic aldehyde (a-HCA) incorporated into sideseam adhesive were analyzed for the amounts of a-HCA present. The MS and SS smoke deliveries of a-HCA were also determined for these cigarettes and the results were reported to R. Comes.

Cast leaf samples containing menthol-released polymers have been analyzed for the amounts of polymer present. Polymers used in the study include poly(1-menthyl-1,1-dimethylallyl carbonate), poly(1-menthyl isopropenyl carbonate) and poly(b-1-menthyl itaconate). A GC injection port hydrolysis method was used to release the menthol from the polymer extracted from the cast leaf with hexane following Polytron homogenization and sonication. Good precision was established for all polymers and recoveries of spiked controls were acceptable.

Hong Kong cigarettes along with samples of pallets, cases, cartons, film overwraps and filters were analyzed using dynamic headspace/GC/MS. In addition, some experimentation was done to observe the effect on cigarettes of being in an atmosphere of various cleaning agents. The results were reported to G. Vilcins.

Samples of rinse water from underground ethanol tanks were examined by purge&trap/GC/MS and the results compared to ordinary tap water. No significant difference was found. In addition, a piece of steel coated with the same material used to line the tanks was soaked in ethanol for several days. Portions of the ethanol were subjected to GC/MS. two "clusters" of peaks were noticed on the GC chromatograms, one cluster composed of toluene and xylene, and the other composed of octenones and/or furans.

Cigarettes from a trailer which caught on fire were analyzed to determine if the cigarettes were contaminated. Cigarette samples were subjected to steam distillation/extraction and analyzed by GC. Two unknown peaks, possibly caused from the burning trailer, were found in these samples and were identified by GC/MS.

Numerous samples of impingers, silica traps, XAD resin tubes and fillers were analyzed for ethanol, water, menthol, nicotine and volatile organic compounds. Various techniques including different sample preparation procedures, GC/TCD, GC/FID and HPLC were used for the analyses. Results of these analyses were used to support Program Grain and Engineering.

PROJECT NUMBER : 6902
PROJECT TITLE : Biochemical Special Investigations
PROJECT LEADER : B. D. Davies
WRITTEN BY : E. A. Crockett
PERIOD COVERED : December, 1991

I. NICOTINE SPECIFIC MONOCLONAL ANTIBODY

- A. **Objective:** Develop a nicotine monoclonal antibody based enzyme-linked immunosorbent assay (ELISA) for the determination of nicotine concentrations in samples of interest to PM.
- B. **Results:** In an effort to optimize the current ELISA assay, experiments were conducted to determine whether or not the incubation periods could be shortened if the microtiter plates were agitated. This would enhance the diffusion process of the antibody to the bottom of the microtiter plate which would possibly shorten the time necessary for the reactions to reach equilibrium. A time course study in which plates were incubated for 15, 30, 45, and 60 minutes with agitation showed that the 45 and 60 minute intervals generated absorbance readings comparable to the control which was incubated for 2 hours without agitation.
- C. **Plans:** No further work is planned at this time.
- D. **Reference:**
Gaines, O. Notebook No. 9093, pp. 101-112.

II. ELECTROPHORETIC TECHNOLOGY

- A. **Objective:** Implement two-dimensional gel electrophoresis fractionation techniques to facilitate the separation of complex protein mixtures.
- B. **Results:** A peptide mapping experiment using asparaginyl endopeptidase and arginyl endopeptidase is being designed to explore the similarities of the 18 proteins isolated from PMT preparations. Control experiments were conducted to test the feasibility of excising protein spots from 2-D gels and re-electrophoresing the proteins directly from the spots into 1-D analytical gels. Two different protein spots were excised from 10 replicate gels and equilibrated in 1-D SDS-PAGE gel sample buffer. Two to five spots from each protein were mashed and then pushed into the wells of a 12% small format 1-D SDS-PAGE gel. Streaky protein bands were seen at the appropriate molecular weight after silver staining the gel. A Coomassie stained gel run under identical conditions showed no detectable protein, therefore the protein levels present on the gel were too low in concentration to consider protein sequencing of individual proteins.

Pullulanase was prepared for 2-D electrophoresis and run on small scale (Daiichi) pH 4-8 first dimension IEF gels and then 10% acrylamide SDS second dimension slab

gels. Most of the protein was present in a cluster of 9-15 proteins which were visible at the expected conditions of approximately 100 kDa and pI 5.5. At higher protein loads, 15-20 other proteins at lower molecular weights and various pI were observed.

Dried hops samples (strains Mt. Hood, Fuggles, and #86807) were prepared for 2-D electrophoresis and run on small scale pH 4-8 IEF gels with 10% acrylamide SDS slab gels. Aliquots of these samples were also run on analytical 1-D SDS-PAGE gels. The 1-D gels showed a heavily streaked sample that also had discrete bands. No differences were seen between the different strains. The 2-D gels showed a very streaky pattern with only discrete spots appearing in the region of the gel containing DNase and RNase which are added to samples prior to fractionation. Control gels that contained hops samples blended with carbamylated protein standards showed a normal pattern for the carbamylated proteins indicating that the fractionations were proceeding normally. No difference was seen between the different strains.

- C. **Plans:** Repeat experiments using different equilibration conditions to improve protein recovery and band resolution. Explore electroelution as a means to recover protein from 2-D gel spots. Prepare hops samples using a methanol extraction to remove potential interfering substances. Rerun pullulanase and hops samples on large scale ISODALT gels.

D. **Reference:**

Krauss, M. R. Notebook No. 9088, pp. 172-189.

III. BIOSENSOR TECHNOLOGY

- A. **Objective:** Develop biosensor technology in-house to allow the determination of the concentration of gas phase compounds of interest.
- B. **Results:** An ImmunoPure (A/G) IgG Purification Kit from Pierce was used to purify the IgG antibody from clone HA4 from other proteins in ascites fluid. However, the protein concentration of the final sample was too low to be used on the biosensor crystals.

Several previously used crystals were washed with concentrated HCl, coated with Protein A, and HA4 in ascites fluid. The crystals were subjected to nicotine in the gas phase. No significant decrease in frequency was observed in any of the crystals.

- C. **Plans:** Conduct gas phase experiments using new crystals and incorporating different techniques as suggested by Dr. G. Guilbault of the University of New Orleans.

D. **Reference:**

Crockett, E. A. Notebook No. 9127, pp. 79-90.

PROJECT NUMBER : 6906
PROJECT TITLE : Biological Effects of Smoke
PROJECT LEADER : G. M. Nixon
PERIOD COVERED : December, 1991

HEME OXYGENASE INDUCTION IN 3T3 AND 3T12 CELLS

- A. **Objective:** To investigate the induction of (putative) heme oxygenase (HO) in 3T3 and 3T12 cells under various conditions.
- B. **Results:** The cycloheximide experiment using 3T12 cells was repeated, with results duplicating those previously reported: HO induction was dependent upon ongoing protein synthesis in all conditions tested, and the 3T12 cells were much less sensitive to any treatment than were the 3T3 cells. A two-dimensional gel experiment using the minigel format and pH 3-10 ampholytes indicated that the basic region of the gel was not being adequately focussed. A carbamylated creatine phosphokinase sample was used to test a large gel format at pH 4-8 and 3-10 ranges. There was insufficient protein on the gels to draw conclusions from this experiment.
- C. **Plans:** Members of Project 6902 are continuing to troubleshoot the 2D process. The manuscript reporting results to this point will undergo final revisions.
- D. **Reference:**

Nixon, G. M. Notebook No. 8711, pp. 179-180.

2057725232